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Phospholipid Transfer between Phosphatidylcholine–Taurocholate Mixed Micelles[†]

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ABSTRACT: The transfer of fluorescent-labeled *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (*N*-NBD-PE) between phosphatidylcholine–taurocholate mixed micelles was measured by monitoring the increase in fluorescence as *N*-NBD-PE, initially contained in mixed micelles at self-quenching concentrations, was diluted into unlabeled mixed micelles. The half-times for transfer of a homologous series of *N*-NBD-PEs differing in saturated acyl chain length from 11 to 16 carbons increased with acyl chain length from 4 to 35 s. The half-times for transfer of the same *N*-NBD-PEs between phosphatidylcholine vesicles without taurocholate were 200–6000 times slower than those between the mixed micelles. A kinetic analysis of initial transfer rate data was used to determine the mechanistic model that best described the data. According to this analysis, the increased rate of intermicellar phospholipid transfer relative to that of intervesicular transfer is a result of (1) exchange between micelles during transient micelle collisions which is not observed between vesicles and (2) an increased rate of monomer diffusion due to a faster rate of phospholipid dissociation from mixed micelles into the water phase than from vesicles. The relative significance of collision-dependent versus monomer diffusion transfer increases with acyl chain length and hydrophobicity.

The release of mixed micelles containing phospholipids, cholesterol, and bile salts from the gall bladder following a fatty meal is essential for the efficient digestion and absorption of fats and other insoluble amphiphiles. Although the thermodynamics of mixed bile salt micelle formation and the factors that determine their size and structure have been studied in detail [for reviews, see Cary and Small (1972), Hofmann and Roda (1984), and Whiting (1986)], virtually nothing is known about the rates and mechanisms by which the water-insoluble components of these micelles are exchanged.

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The transfer of phospholipids has been measured between phospholipid vesicles (Martin & McDonald, 1976; Papahadjopoulos et al., 1976; Duckwitz-Peterlein et al., 1977; Kremer et al., 1977a; Roseman & Thompson, 1980; Nichols & Pagano, 1981, 1982; McLean & Phillips, 1981, 1984; DeCuyper et al., 1983, 1985), between lipoproteins (Jonas & Maine, 1979; Massey et al., 1982a,b, 1984; Petrie & Jonas, 1984), and between cellular membranes (Stuhne-Sekalec & Stanacev, 1982; Rindlisbacher & Zahler, 1983; Ferrell et al., 1985; Mütsch et al., 1986). In most of the systems studied, spontaneous phospholipid transfer has been shown to occur by soluble monomer diffusion through the aqueous phase. However, in a few cases, transfer appears to occur by a collision-dependent mechanism (Jonas & Maine, 1979; Stuhne-Sekalec & Stanacev, 1982; Petrie & Jonas, 1984; Mütsch et al., 1986).

In the present study, fluorescent-labeled *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (*N*-NBD-PE)[1] molecules were shown to transfer 200–6000 times faster between phosphatidylcholine–taurocholate mixed micelles than between phospholipid vesicles. A kinetic analysis indicated that the increased rate of intermicellar transfer resulted from phospholipid exchange during transient micelle collisions and a concomitant increase in the rate of phospholipid dissociation into the water phase, the rate-limiting step for monomer diffusion. A preliminary report of this work has appeared elsewhere (Nichols, 1988).

EXPERIMENTAL PROCEDURES

Materials and Routine Procedures. PC, DUPC, DTPC, DOPE, DLPE, DMPE, and DPPE were purchased from Avanti Polar Lipids, Inc. Sodium taurocholate, NBD-Cl, and phospholipase D (cabbage) were purchased from Sigma Chemical Co. Lissamine rhodamine B sulfonyl chloride was purchased from Molecular Probes, Inc. DUPE and DTPE were prepared from their respective phosphatidylcholine analogues by phospholipase D catalyzed base exchange (Comfurius & Zwaal, 1977). DOPE was reacted with lissamine rhodamine B sulfonyl chloride to yield *N*-Rh-PE, and the saturated diacylphosphatidylethanolamines were reacted with NBD-Cl to yield a series of *N*-NBD-PE molecules. Synthesis and purification were performed as described by Struck et al. (1981). Sodium taurocholate was tested for purity by silica gel thin-layer chromatography. Densitometric analysis of the plates following sulfuric acid charring indicated greater than 99% purity. Phospholipids were stored at −20 °C, periodically monitored for purity by thin-layer chromatography, and repurified when necessary. Phospholipid concentrations were determined by a lipid phosphorus assay (Ames & Dubin, 1960).

Mixed Micelle and Vesicle Preparation. Mixed micelles were prepared by evaporating the solvents from equimolar amounts of phospholipid and TC under nitrogen followed by overnight desiccation in vacuo. The dried phospholipid–TC mixtures were solubilized in HBS containing additional TC to obtain the desired mole ratio and concentrations of phospholipid and TC. Mixtures were stirred until clear and no further reduction in light-scattering could be detected. Phospholipid vesicles were prepared by ethanol injection as described previously (Kremer et al., 1977b; Nichols & Pagano, 1982).

Fluorescence and Light Scattering Measurements. Fluorescence and light scattering were measured with a Perkin-Elmer MPF-44E fluorescence spectrophotometer. Solutions in the cuvette were stirred continuously by a magnetic stirrer, and the temperature was maintained constant at 25 °C by a circulating water bath. Inner filtering was minimized by use of *N*-NBD-PE concentrations that resulted in less than 0.2 absorbance at the excitation maximum wavelength. For the fluorometer settings used in these experiments, no fluorescence signal was detected from a blank cuvette containing the highest concentration of PC–TC (6:12, mM) micelles in HBS. The analogue output from the fluorometer was digitized and stored on an IBM-XT.

Measurement of N-NBD-PE Transfer between Micelles. The transfer of *N*-NBD-PE from donor micelles containing *N*-NBD-PE at self-quenching concentrations to unlabeled acceptor micelles was determined from the fluorescence increase observed following mixing. Equilibration of the *N*-NBD-PE between the donor and acceptor micelles reduced the concentration-dependent self-quenching, resulting in an increase in fluorescence (Figure 2).

For all of the *N*-NBD-PEs and for all concentrations of donor and acceptor micelles tested in these experiments, the rate of fluorescence increase observed following the mixing of donor and acceptor micelles could be accurately described by a first-order exponential equation (see Figure 2). In order to fit these data with the initial rate equations derived below for the different mechanisms of transfer, the apparent first-order rate constant (k_{app}) obtained from the nonlinear least-squares fit of the fluorescence data was used to calculate the initial rate of fluorescence change:

$$\frac{\Delta F_{t=0}}{\Delta t} = k_{app}\Delta F_{eq} \tag{1}$$

where $\Delta F_{t=0}/\Delta t$ is the initial rate of fluorescence change and ΔF_{eq} is the fluorescence change at equilibrium. Dividing the initial rate by the maximum change in fluorescence (ΔF_{max}; the maximum fluorescence increase obtained following equilibration with excess acceptor micelles) expresses the initial rate as the fractional rate of maximum fluorescence:

$$\frac{\Delta F_{t=0}}{\Delta F_{max}\Delta t} = \frac{k_{app}\Delta F_{eq}}{\Delta F_{max}} \tag{2}$$

The initial fractional rate of maximum fluorescence is equal to the initial fractional rate of maximum *N*-NBD-PE transfer from donor to acceptor micelles (this relationship is derived below, eq 14):

$$\frac{\Delta F_{t=0}}{\Delta F_{max}} = \frac{(\Delta[\mathrm{D}]_2)_{t=0}}{[\mathrm{D}]_\mathrm{T}} \tag{3}$$

Combining eq 2 and 3 gives

$$R = \frac{(\Delta[\mathrm{D}]_2)_{t=0}}{[\mathrm{D}]_\mathrm{T}\Delta t} = \frac{k_{app}\Delta F_{eq}}{\Delta F_{max}} \tag{4}$$

Initial fractional rates of maximum transfer (R, s^{-1}) were calculated for each experimental trial and used in the kinetic analysis described under Results.

Correlation of Fluorescence Transients with Intermicellar N-NBD-PE Transfer. The relationship of the fluorescence yield (*f*) of *N*-NBD-PE to its mole fraction concentration in

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[1] Abbreviations: IMC, intermicellar concentration; PC, dioleoylphosphatidylcholine; TC, taurocholate; DUPC, diundecanoylphosphatidylcholine; DTPC, ditridecanoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DUPE, diundecanoylphosphatidylethanolamine; DLPE, dilaurylphosphatidylethanolamine; DTPE, ditridecanoylphosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; *N*-NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)diacylphosphatidylethanolamine; HBS, HEPES-buffered saline, 0.9% NaCl in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4.

PC–TC micelles can be described by the Stern–Volmer equation (data not shown):

$$f = \frac{f_{\max}}{1 + K[\mathrm{D}]/[\mathrm{L}]} \qquad (5)$$

where f is the fluorescence yield (RFU/mol), [D] is the bulk solution concentration of micellar *N*-NBD-PE, [L] is the bulk solution concentration of total phospholipid in the micelles, $f_{\max}$ is the maximum fluorescence yield for *N*-NBD-PE contained in PC–TC micelles, and K is the quenching constant. The total fluorescence observed following the mixing of donor and acceptor micelles (F) is equal to the sum of the fluorescence in the donor micelles (F_1) and the acceptor micelles (F_2), which are both equal to the product of the bulk solution concentrations of *N*-NBD-PE in each of the micelle populations and their respective fluorescence yields. Thus

$$F = F_1 + F_2 = [\mathrm{D}]_1 f_1 + [\mathrm{D}]_2 f_2 \qquad (6)$$

The change in the fluorescence (ΔF) observed at a given time (t) following the mixing of donor and acceptor micelles is equal to the difference in the fluorescence observed at time t and the fluorescence observed immediately following mixing (F_0), where $t = 0$:

$$\Delta F = F - F_0 \qquad (7)$$

According to the experimental protocol used in these experiments, the total concentration of *N*-NBD-PE ($[\mathrm{D}]_\mathrm{T}$) is initially contained in the donor micelles; thus, at $t = 0$, $[\mathrm{D}]_1 = [\mathrm{D}]_\mathrm{T}$, $[\mathrm{D}]_2 = 0$, and

$$F_0 = \frac{[\mathrm{D}]_\mathrm{T} f_{\max}}{1 + K[\mathrm{D}]_\mathrm{T}/[\mathrm{L}]_1} \qquad (8)$$

Since the amount of *N*-NBD-PE dissolved in the water phase is small relative to that associated with micelles

$$[\mathrm{D}]_\mathrm{T} \approx [\mathrm{D}]_1 + [\mathrm{D}]_2 \qquad (9)$$

Thus, eq 5–9 can be combined to describe the relationship between the observed change in fluorescence following the mixing of donor and acceptor micelles and the transfer of *N*-NBD-PE from donor to acceptor micelles ($\Delta[\mathrm{D}]_2$):

$$\Delta F = \frac{([\mathrm{D}]_\mathrm{T} - \Delta[\mathrm{D}]_2) f_{\max}}{1 + K([\mathrm{D}]_\mathrm{T} - \Delta[\mathrm{D}]_2)/[\mathrm{L}]_1} + \frac{\Delta[\mathrm{D}]_2 f_{\max}}{1 + K\Delta[\mathrm{D}]_2/[\mathrm{L}]_2} - \frac{[\mathrm{D}]_\mathrm{T} f_{\max}}{1 + K[\mathrm{D}]_\mathrm{T}/[\mathrm{L}]_1} \qquad (10)$$

Rearranging the terms

$$\Delta F = [\mathrm{D}]_\mathrm{T} f_{\max}\left\{\frac{[\mathrm{L}]_1}{[\mathrm{L}]_1 + K([\mathrm{D}]_\mathrm{T} - \Delta[\mathrm{D}]_2)} - \frac{[\mathrm{L}]_1}{[\mathrm{L}]_1 + K[\mathrm{D}]_\mathrm{T}}\right\} + \Delta[\mathrm{D}]_2 f_{\max}\left\{\frac{[\mathrm{L}]_2}{[\mathrm{L}]_2 + K\Delta[\mathrm{D}]_2} - \frac{[\mathrm{L}]_1}{[\mathrm{L}]_1 + K([\mathrm{D}]_\mathrm{T} - \Delta[\mathrm{D}]_2)}\right\} \qquad (11)$$

For the initial rate of fluorescence change $\Delta F_{t=0}$, when $\Delta[\mathrm{D}]_2$ is very small, eq 11 reduces to

$$\Delta F_{t=0} = (\Delta[\mathrm{D}]_2)_{t=0} f_{\max}\left(1 - \frac{[\mathrm{L}]_1}{[\mathrm{L}]_1 + K[\mathrm{D}]_\mathrm{T}}\right) \qquad (12)$$

The maximum change in fluorescence ($\Delta F_{\max}$) occurs at equilibrium in the presence of excess acceptor micelles ($[\mathrm{L}]_2 \gg [\mathrm{L}]_1$) such that $\Delta[\mathrm{D}]_2 \approx [\mathrm{D}]_\mathrm{T}$. For this condition eq 11 reduces to

$$\Delta F_{\max} = [\mathrm{D}]_\mathrm{T} f_{\max}\left(1 - \frac{[\mathrm{L}]_1}{[\mathrm{L}]_1 + K[\mathrm{D}]_\mathrm{T}}\right) \qquad (13)$$

Thus, dividing eq 12 by eq 13 yields

$$\frac{\Delta F_{t=0}}{\Delta F_{\max}} = \frac{(\Delta[\mathrm{D}]_2)_{t=0}}{[\mathrm{D}]_\mathrm{T}} \qquad (14)$$

The initial fractional change of the maximum fluorescence is equal to the initial fractional amount of total *N*-NBD-PE transferred from donor to acceptor micelles.

Kinetic Models for Phospholipid Transfer between Micelles. There are two kinetically distinguishable mechanisms for the transfer of lipids between micelles: (1) lipid transfer occurs by the diffusion of monomers through the aqueous phase, and (2) lipid transfer is dependent upon micelle–micelle collision or fusion. Rate equations for both mechanisms can be written on the basis of the principles of mass action kinetics. These equations have been derived elsewhere for lipid transfer between micelles (Nakagawa, 1974) and between phospholipid vesicles or cell membranes (Thilo, 1977; Nichols & Pagano, 1981, 1982; Ferrell et al., 1985). Given the special experimental case in which the donor and acceptor micelles are identical, the initial rate equations can be simplified, such that the monomer diffusion mechanism is predicted by

$$\left(\frac{\Delta[\mathrm{D}]_2}{\Delta t}\right)_{t=0} = \frac{k_{\mathrm{dis}}[\mathrm{D}]_1[\mathrm{L}]_2}{[\mathrm{L}]_1 + [\mathrm{L}]_2} \qquad (15)$$

or since at $t = 0$ $[\mathrm{D}]_1 = [\mathrm{D}]_\mathrm{T}$, eq 15 can be expressed as the fractional rate of maximum transfer (R):

$$R = \frac{k_{\mathrm{dis}}[\mathrm{L}]_2}{[\mathrm{L}]_1 + [\mathrm{L}]_2} \qquad (16)$$

where R is the fractional rate of probe D transfer from donor to acceptor micelles, k_{dis} is the dissociation rate constant for probe D leaving the micelles, and $[\mathrm{D}]_1$, $[\mathrm{L}]_1$, and $[\mathrm{L}]_2$ are the bulk solution concentrations of probe in the donor micelles, the concentration of phospholipid in the donor micelles, and the concentration of phospholipid in the acceptor micelles, respectively.

Micelle collision-dependent transfer can occur upon the collision or transient fusion of two micelles (bimolecular) or of three micelles (termolecular). The bimolecular collision-dependent transfer mechanism is predicted by

$$\left(\frac{\Delta[\mathrm{D}]_2}{\Delta t}\right)_{t=0} = k_{\mathrm{bi}}[\mathrm{D}]_1[\mathrm{L}]_2 \qquad (17)$$

or as the fraction rate

$$R = k_{\mathrm{bi}}[\mathrm{L}]_2 \qquad (18)$$

and the termolecular collision-dependent transfer mechanism by

$$\left(\frac{\Delta[\mathrm{D}]_2}{\Delta t}\right)_{t=0} = k_{\mathrm{ter}}[\mathrm{D}]_1([\mathrm{L}]_2)^2 \qquad (19)$$

or

$$R = k_{\mathrm{ter}}([\mathrm{L}]_2)^2 \qquad (20)$$

where k_{bi} is the apparent bimolecular rate constant and k_{ter} is the apparent termolecular rate constant.

Data Analysis. Fits of the time-based fluorescence data and the initial rate data versus acceptor micelle concentration were made with a nonlinear least-squares program based on the Marquardt algorithm (Marquardt, 1963). The F_χ test was used to test the significance of the addition of terms for im-

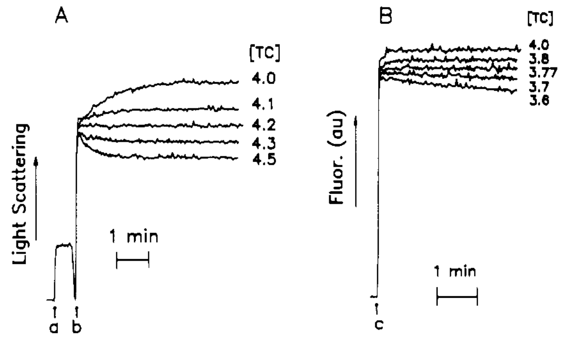

FIGURE 1: Measurement of the intermicellar taurocholate concentration for phosphatidylcholine–taurocholate mixed micelles. (A) The intensity of Rahleigh scattered light (90°, λ = 450 nm; band-pass: 1 nm, entering; 4 nm, exiting) for a series of TC solutions in HBS (2-mL volume, concentration in millimolar is indicated for each trace) was measured before (a) and after (b) the addition of 0.2 mL of PC–TC mixed micelles (10 mM PC; 14.2 mM TC). The intensity of light scattering of an HBS solution [trace prior to (a)] is not significantly different from the fluorometer dark current. (B) The fluorescence intensity in arbitrary units (excitation 475 nm, 2-nm slit width; emission 530 nm, 2-nm slit width) was recorded following the addition (c) of 0.01 mL of mixed micelles [*N*-NBD-DLPE:PC:TC, 1.0:4.0:9.2 (mM)] to a cuvette containing 2 mL of HBS with different concentrations of TC. Concentrations in millimolar are indicated for each trace. The fluorescence of the TC solutions in HBS [trace prior to (c)] is not significantly different from the dark current. Temperature 25 °C.

proving the value of the variance [see p 200 in Bevington (1969)]. F_χ is defined as

$$F_\chi = \frac{\chi^2(n-1) - \chi^2(n)}{\chi^2(n)/(N-n-1)} \tag{21}$$

where $\chi^2(n)$ is χ-square for n terms, N is the number of data points, and n is the number of terms. This statistic follows the F distribution with one degree of freedom and can be used to test the null hypothesis that the coefficient of the added term is equal to zero (i.e., the new term does not improve the goodness of fit).

RESULTS

Measurement of the Intermicellar Concentration of Taurocholate. Dilution of a PC–TC micellar mixture with buffer results in an increase in the ratio of PC to TC in the micelles because a larger amount of TC partitions out of the micelles into the newly added water phase than PC. Since the size and number of PC–TC mixed micelles is dependent on the ratio of the two components in the micelle (Mazer et al., 1980), care must be taken to avoid changes in the average micellar disk diameter while varying the total micelle concentration. By diluting the PC–TC solutions with buffer containing TC at the intermicellar concentration (IMC) of the mixture, the ratio of PC to TC in the micelles remains constant, and the total concentration can be varied without affecting the micelle size (Mazer et al., 1980).

For the transfer experiments presented here, donor (containing *N*-NBD-PE) and acceptor micelles were prepared with the same concentrations and ratios of phospholipid and TC, and the donor and acceptor IMCs for TC were found to be equal. Changes in light scattering (Figure 1A) and fluorescence (Figure 1B) were used to determine the IMCs for acceptor and donor micelles, respectively. At "b" in Figure 1A the intensity of 90° scattered light is recorded following the addition of the same amount of mixed PC–TC micelles to different concentrations of TC in HBS. Following the initial increase in the intensity of scattered light, there is a transient decrease in intensity at concentrations of TC above 4.2 mM, and a transient increase at concentrations below 4.2. These transients were interpreted according to the "mixed-disk" model of Mazer et al. (1980) to reflect changes in micelle particle size as a function of the PC:TC ratio. At concentrations of TC greater than the IMC, TC partitions into the micelles, decreasing the PC:TC ratio and decreasing the particle size and intensity of light scattering. At concentrations of TC below the IMC, the reverse occurs. This technique was used to determine the IMC to within 0.1 mM.

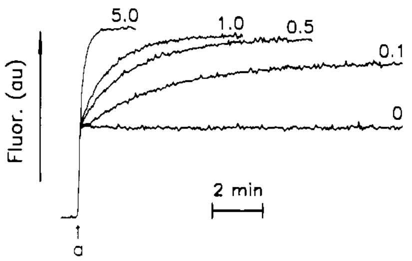

FIGURE 2: Series of fluorescence traces reflecting the transfer of *N*-NBD-DMPE from donor to acceptor mixed micelles. Fluorescence (excitation 475 nm; emission 530 nm; slit widths 2 nm) was recorded as 0.01 mL of donor micelles [*N*-NBD-DMPE:PC:TC, 2:8:20 (mM)] was added to 2 mL of 4.3 mM TC in HBS containing varying amounts of acceptor micelles [PC:TC 10:20 (mM)]. Each trace is labeled with the final concentration (mM) of PC in the cuvette. The fluorescence of 4.3 mM TC in HBS [trace prior to (a)] is not significantly different from the dark current. Temperature 25 °C.

The expense of the fluorescent probes prohibited the preparation of the large amounts of mixed micellar solutions required to measure changes in light scattering. However, the self-quenching interaction of the *N*-NBD-PE probes is very sensitive to their concentration in the micelles, which allows for the detection of small changes in the TC content of the micelles. Thus, NBD fluorescence could be used to determine the IMC for these micelles, as illustrated in Figure 1B. At "c" in Figure 1B, the NBD fluorescence is recorded following the addition of the same amount of donor micelles into different concentrations of TC in HBS. Following the initial rapid fluorescence increase, the fluorescence decreases at TC concentrations below, and increases at concentrations above, 3.77 mM. These transients were interpreted to reflect changes in the ratio of TC to *N*-NBD-PE in the micelles. At concentrations above the IMC, TC partitions into the micelles, reducing the mole fraction of *N*-NBD-PE in the micelles which reduces the extent of self-quenching. The reverse occurs at TC concentrations below the IMC. TC is removed from the micelles, increasing the mole fraction of probe in the micelles, which increases the self-quenching and decreases fluorescence. By use of this technique, the IMC for low concentrations of the *N*-NBD-PE-containing mixed micelles was detected to within a few hundredths of a millimolar.

For the experiments presented in Figure 3 and in Table I, the donor and acceptor micelles were prepared with identical molar ratios of phospholipid (*N*-NBD-PE and/or PC) to TC which resulted in an identical IMC (4.3 mM) for both the donor and acceptor micelles. Therefore, concentrations of acceptor micelles were adjusted with this concentration of TC in HBS.

N-NBD-PE Transfer between Micelles. A series of fluorescence traces illustrating the transfer of *N*-NBD-DMPE between PC–TC micelles is presented in Figure 2. At "a" in Figure 2, donor micelles containing *N*-NBD-DMPE are injected into a stirred fluorescence cuvette containing different concentrations of acceptor micelle PC (numbers above traces in millimolar). The lower trace (0 mM) demonstrates that, following the initial rapid fluorescence increase, no subsequent

Table I: Compilation of Rate Constants for the Comparison of *N*-NBD-PE Transfer between PC–TC Mixed Micelles and between PC Vesicles

		intermicelle transfer[a]				intervesicle transfer[b]	
N-NBD-PE	acyl chain length	$t_{1/2}$ (min)[c]	k_{dis} (s^{-1})	k_{bi} ($M^{-1} \cdot s^{-1}$)[d]	k_{ter} ($M^{-2} \cdot s^{-1}$)[d]	$t_{1/2}$ (min)	k_{dis} (s^{-1})
DUPE	11	0.063	0.19 (±3.0 × 10^{-3})[e]			14	8.2 × 10^{-4}
DLPE	12	0.17	3.3 × 10^{-2} (±1.0 × 10^{-3})	12.0 (±1.0)	1.2 × 10^{3} (±1.7 × 10^{2})	137	8.4 × 10^{-5}
DTPE	13	0.38	7.5 × 10^{-3} (±5.6 × 10^{-4})	8.6 (±0.55)	1.2 × 10^{3} (±94.0)	1190	9.7 × 10^{-6}
DMPE	14	0.45	3.4 × 10^{-3} (±5.1 × 10^{-4})	7.8 (±0.50)	1.3 × 10^{3} (±85.0)	2800	4.1 × 10^{-6}
DPPE	16	0.58	1.8 × 10^{-3} (±6.3 × 10^{-4})[f]	7.6 (±0.61)	6.5 × 10^{2} (±1.0 × 10^{2})		

[a] Intermicelle transfer was measured as illustrated in Figure 2. Rate constants were obtained from nonlinear least-squares fits of the initial rate data (Figure 3) as described under Experimental Procedures. All measurements were made at 25 °C. [b] Intervesicle transfer was measured as described under Results. [c] Half-times were calculated from k_{app} obtained at 2 mM acceptor micelle PC. [d] M units refer to the bulk solution concentration of PC in the acceptor micelles. [e] Numbers in parentheses are the standard errors of the fit parameters calculated according to Bevington (1969), p 242. [f] Applying the F_{χ} test (eq 21), the probability that this term is not equal to zero when added to the remaining two terms in the rate equation is greater than 95%. For each of the remaining intermicellar rate constants, the probability is greater than 99% that they are not equal to zero when added as the third term in the sum of rate equations 16, 18, and 20.

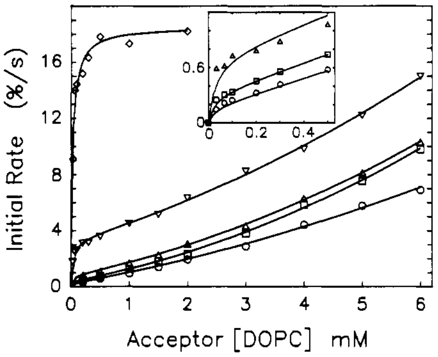

FIGURE 3: Plot of the initial fractional rate of maximum transfer from donor to acceptor micelles for a series of *N*-NBD-PE probes varying in acyl chain length versus the concentration of acceptor micelle phosphatidylcholine. Rate measurements were performed as illustrated in Figure 2. Initial rates were calculated from eq 5 under Results and expressed as percent transfer (R × 100). Donor micelles were composed of *N*-NBD-PE:PC:TC [2:8:20 (mM)] in HBS, and acceptor micelles contained PC:TC [10:20 (mM)] in HBS. Symbols refer to *N*-NBD-DUPE (◇), *N*-NBD-DLPE (▽), *N*-NBD-DTPE (△), *N*-NBD-DMPE (□), and *N*-NBD-DPPE (○). Solid lines were generated from the sums of eq 16, 18, and 20 with the best-fit parameters presented in Table I. Inset expands data at low concentrations. Temperature 25 °C.

change in fluorescence is observed when donor micelles containing *N*-NBD-DMPE are diluted into a solution of 4.3 mM TC in the absence of mixed PC–TC micelles. This indicates that the mole fraction of *N*-NBD-DMPE in the donor micelles is unchanged and confirms that 4.3 mM is the IMC for these donor micelles. Injection of the same amount of donor micelles into solutions containing mixed PC–TC micelles results in a subsequent increase in fluorescence.

Kinetic Analysis of N-NBD-PE Transfer between Micelles. The initial rates of intermicellar transfer for a homologous series of *N*-NBD-PE molecules with saturated diacyl chains varying from 11 to 16 carbons were measured and plotted as a function of acceptor micelle PC concentration (symbols, Figure 3). The data points for a given probe were fit to each of the three transfer models (eq 16, 18, and 20). The kinetics of transfer of *N*-NBD-DUPE (diamonds in Figure 2) were best fit by the monomer diffusion model alone (eq 16). However, the kinetics of transfer for the remaining four probes were too complex to be described by any of the three models alone. If one assumes that both monomer diffusion and collision-dependent mechanisms are occurring simultaneously and independently, the initial rates will be equal to the sum of eq 16, 18, and 20. Thus, the best fit of initial rate data may be obtained for an initial rate equation that includes one, two, or all three of the right-hand terms of eq 16, 18, and 20, depending on which combination of mechanisms is involved in the transfer. The minimum number of terms necessary to describe the data was determined as follows. The data for a given probe were fit to each of the three models to determine the model which gave the best fit (minimum χ^2). The F_{χ} test was used to determine if addition of either of the two reamining terms significantly improved the goodness of fit. Finally, the F_{χ} test was used to determine if addition of the third term significantly improved the fit. With the exception of *N*-NBD-DUPE, the summation of the terms for all three mechanisms resulted in significant improvement of the fit (see legend of Table I for statistics) of the kinetics of the remaining four probes. The theoretical projections of the initial rate equations are plotted as solids lines in Figure 3, and the best-fit parameters are presented in Table I.

These kinetic data indicate that the predominant mechanism for intermicellar transfer of the short-chain *N*-NBD-DUPE at these PC–TC concentrations is by the diffusion of soluble monomers through the water phase. For the remaining four probes, the relative sizes of the three terms of the rate equation indicate that at low concentrations of acceptor micelles monomer diffusion is the predominant mode of intermicellar transfer, whereas at higher concentrations bi- and termolecular collision-dependent transfer predominates. These collision or fusion complexes are transient since no increase in light scattering of the micellar solutions is observed during the transfer experiments. Presumably, fusion of two micelles destabilizes the balance of energetic forces and geometric constraints that determines the micellar size, and the fused micelle rapidly divides to return to a more stable size.

Comparison of Micelle–Micelle Transfer with Vesicle–Vesicle Transfer. Resonance energy transfer between *N*-NBD-PE and head-group-labeled rhodamine-PE (Nichols & Pagano, 1982) was used to measure the transfer of the homologous series of *N*-NBD-PE probes between phospholipid vesicles. Transfer of all of the *N*-NBD-PE probes was a first-order exponential process, and the half-times were independent of the vesicle concentration (data not shown) indicating that transfer occurs by the diffusion of soluble monomers through the water phase (Nichols & Pagano, 1982). Under these conditions, the first-order rate constant is equal to the dissociation rate constant for *N*-NBD-PE monomer transfer from the vesicle to water phase. Half-times and dissociation rate constants are presented in Table I.

These data indicate that, for the four probes where intervesicle transfer data were obtained, transfer between PC–TC bile salt micelles (at 2 mM micellar DOPC) occurs between 200 and 6000 times faster than that between PC vesicles. For probes with longer acyl chains (*N*-NBD-DTPE and -DMPE), the increased rate of transfer results predominantly from the exchange of molecules during transient micelle collisions or fusions which does not occur between vesicles, whereas the increased rates of intermicellar relative to intervesicular transfer observed for the probes with shorter acyl chains (*N*-NBD-DUPE and -DLPE) are predominantly the result of faster monomer dissociation from micelles into the water phase. However, the micelle dissociation rate constant for all the probes is from 200 to 800 times greater than those measured for vesicles.

DISCUSSION

These are the first measurements of the transfer of phospholipids between phospholipid–bile salt mixed micelles. The kinetic analysis of these data demonstrated two previously undescribed properties of mixed micelles that explain the observed rapid intermicellar transfer of phospholipids relative to intervesicular transfer: (1) transient collisions or fusions which allow for rapid exchange of phospholipids and presumably other hydrophobic molecules and (2) a faster rate of phospholipid dissociation from the mixed micelles than from phospholipid vesicles.

In order to understand better their meaning, the bimolecular and termolecular rate constants presented in Table I can be expressed as the products of several parameters and rate constants that describe the individual events involved in the successful collision and transfer of phospholipids:

$$k_{\mathrm{bi}} = \frac{k_c \alpha \beta_1}{n_L^2}$$

and

$$k_{\mathrm{ter}} = \frac{(k_c)^2 \alpha \beta_2}{n_L^3}$$

where k_c is the diffusion-limited collisional rate constant, α is the fraction of phospholipid molecules exchanged during the lifetime of a transient fusion complex, β_1 is the fraction of micelle collisions that result in a transient fusion complex capable of exchanging phospholipids, β_2 is the fraction of micelle collisions with a transient fusion complex that results in a termolecular fusion complex capable of exchanging phospholipids, and n_L is the number of phospholipid molecules per micelle.

The quasi-elastic light scattering data of Mazer et al. (1980) indicate that the average radius of mixed-disk micelles for the ratio of PC to TC used in these experiments is 1.6 nm. The mixed-disk model proposes that PC forms a bilayer disk which is saturated with TC dimers and surrounded by TC monomers. According to this model, the average micelle in these experiments contains 30 PC molecules (n_L) and 44 TC molecules. Assuming that all phospholipids exchange during the lifetime of a transient fusion complex ($\alpha = 1$) and that the frequency of micellar collison is diffusion-limited ($k_c = 10^9$ M^{-1} s^{-1}; Gardner, 1969), the maximum fraction of collisions that result in the formation of a transient fusion complex can be calculated from the bi- and termolecular rate constants: $\beta_1 = 7 \times 10^{-6}$ and $\beta_2 = 4 \times 10^{-6}$. In other words, one out of every 140 000 micelle collisions results in a transient fusion complex that is capable of exchanging phospholipids; and following complex formation, one out of every 250 000 collisions of a micelle with the complex results in a termolecular complex capable of exchanging phospholipids. According to the Eyring rate theory, the free energy of activation for the formation of the bimolecular collision complex between two micelles ($\Delta G^{\ddagger\circ}$) is 12.2 kcal/mol at 25 °C.

The observation that the bi- and termolecular rate constants are relatively independent of acyl chain length (Table I) suggests that the exchange of phospholipids during the transient fusion complexes does not require exposure of the hydrophobic acyl chains of the phospholipids to the water. Formation of these transient fusion complexes may provide one component of the "hydrocarbon continuum" that has been postulated to explain the intestinal absorption of very hydrophobic lipid molecules (Patton, 1981).

The micellar dissociation rate constant for the probes for which comparable data were obtained ranged from 200 to 800 times faster than that from vesicles. The rapid dissociation of phospholipid from the mixed-disk micelles may be due to the ability of phospholipid molecules to dissociate from the edge of the disk, whereas phospholipids must exit from bilayer vesicles in a path that is normal to the plane of the bilayer. According to the mixed-disk model, the acyl chains of the phospholipids and the periphery of the disk are protected from exposure to water by a single layer of bile salt molecules. However, one would predict from the hydrophobicity of bile salts that their exchange between micelles is very rapid (Aniansson et al., 1976; Phillips et al., 1987). Although in the steady state bile salts are hydrophobically bound to the periphery of the disk, their rapid turnover continually exposes the acyl chains of the peripheral phospholipids to water, increasing their free energy in the micelle. Thus, less activation free energy is required to transfer the phospholipids at the disk periphery into the water phase. This low-energy pathway is not available for the dissociation of phospholipids from vesicles.

Mixed-disk phospholipids may be further destabilized by the inability of bile salts to shield the entire length of the phospholipid acyl chains at the periphery of the disks. From space-filling models, the length of the hydrophobic steroid nucleus of bile salts is approximately equivalent to a 13-carbon acyl chain. Thus, the length of the bile salt molecules surrounding the phospholipid disk is insufficient to protect completely the phospholipids with acyl chains longer than 13 carbons from association with water. This may further increase the micellar free energy of phospholipids with long acyl chains and further increase their dissociation rate.

From these kinetic data it is not possible to determine the equilibrium constants for water-soluble monomer–micelle and water-soluble monomer–vesicle interactions. However, since the rate of phospholipid monomer association with vesicles is very nearly, if not determined by, the rate of aqueous diffusion (McLean & Phillips, 1984; Nichols, 1985), the rate of monomer association with micelles is most likely also limited by diffusion. If it is assumed that the association rate constants are diffusion-limited, the ratio of affinity constants for monomer to vesicle association and monomer to micelle association ($K_{\mathrm{ves}}/K_{\mathrm{mic}}$) will be equal to the inverse of the ratio of the dissociation rate constants ($k_{\mathrm{dis}}^{\mathrm{mic}}/k_{\mathrm{dis}}^{\mathrm{ves}}$). Thus, depending on the probe, the equilibrium constant for a monomer associating with a PC vesicle bilayer is predicted to be 200–600 times greater than that for a mixed PC–TC micelle. The lower affinity of phospholipids for mixed phospholipid–bile salt micelles relative to that of phospholipid bilayers may greatly facilitate the rapid and complete transfer of phospholipid monomers from mixed micelles to the plasma membrane of enterocytes in the intestinal mucosa.

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Registry No. PC, 10015-85-7; TC, 81-24-3; DOPE, 2462-63-7; DUPE, 114094-50-7; DLPE, 42436-56-6; DTPE, 86388-23-0; DMPE, 20255-95-2; DPPE, 3026-45-7.

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