Spontaneous Interbilayer Transfer of Phospholipids: Dependence on Acyl Chain Composition†

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ABSTRACT: The diffusion-mediated intervesicle transfer of a variety of fluorescent *N*-(*S*-bimanylmercaptosuccinyl)phosphatidylethanolamines (BMS-PEs), and of a more limited series of radiolabeled phosphatidylcholines (PCs), has been investigated as a function of the acyl chain composition of the labeled molecules and the composition of the lipid vesicles. Increasing the total acyl chain length of a BMS-PE by two methylene residues increases the free energy of activation for probe desorption from a lipid bilayer (ΔG_d^*) by ca. 1300 cal mol^{-1}, while the addition of one double bond to either chain decreases ΔG_d^* by a slightly smaller increment. The values of these increments in ΔG_d^* are surprisingly consistent with variations in BMS-PE acyl chain length, positional isomerism (for heteroacyl BMS-PEs), and the sterol content or degree of acyl chain saturation of the lipid bilayer. However, the successive addition of multiple *cis* double bonds to the same acyl chain leads to progressively smaller decrements in the value of ΔG_d^*. Radiolabeled PCs show somewhat lower absolute rates of interbilayer transfer than the corresponding BMS-PEs, but the incremental contributions of added methylene groups or *cis* double bonds to ΔG_d^* are essentially identical for the two classes of lipids, validating the use of BMS-PEs as representative probes of phospholipid behavior for these experiments. Our data suggest that the relative rates of spontaneous transfer of a variety of phospholipids from bilayer membranes can be predicted to good approximation from a few basic features of the phospholipid structure. However, we also conclude that this relationship is quantitatively and, in some regards, even qualitatively very different from that recently proposed by Pownall *et al.* [(1991) *Biochemistry 30*, 5696–5700] from measurements of the transfer rates of phospholipids in a model lipoprotein system.

The spontaneous interbilayer transfer of phospholipids is a relatively slow process, which in biological systems must normally be accelerated by agents such as phospholipid transfer proteins, or augmented by parallel mechanisms such as intracellular "membrane traffic", in processes requiring efficient intermembrane lipid transport (Pagano & Sleight, 1985; Helmkamp, 1986; Dawidowicz, 1987; van Meer, 1989; Voelker, 1989; Bankaitis *et al.*, 1990; Moreau *et al.*, 1991). However, in some biological contexts these low rates of spontaneous intermembrane phospholipid transfer may become significant, as in cases where phospholipid-anchored macromolecules or drug conjugates must remain stably associated with liposomes *in vivo*, or a phospholipid-anchored protein must remain stably bound to a cell surface, for long periods of time (Heath & Martin, 1986; Cross, 1990; Woodle & Lasic, 1992; van Wijk *et al.*, 1992; Silvius & Zuckermann, 1993). For such reasons, it is important to understand how the rate of spontaneous interbilayer transfer of phospholipids can vary with the lipid structure and with the nature of the bilayer membrane environment.

Most previous studies of the spontaneous transfer of phospholipids and phospholipid derivatives between lipid bilayers have focused on the mechanism(s) of the transfer process and on the effects of varying acyl chain length and headgroup structure on the kinetics of transfer [McLean & Phillips, 1981; Nichols & Pagano, 1981, 1982; Ferrell *et al.*, 1985; Nichols, 1985; Homan & Pownall, 1988; Jones & Thompson, 1989, 1990; Wimley and Thompson (1990) and references therein]. No study to date has explored systematically the effect of variables such as acyl chain unsaturation, positional isomerism, and acyl chain mismatch on the rates of spontaneous interbilayer transfer of phospholipids. To explore these questions, we have utilized fluorescent *N*-[2-(*S*-bimanylthio)succinyl]phosphatidylethanolamines (BMS-PEs)[1] and radiolabeled phosphatidylcholines to assess the effects of acyl chain length, unsaturation, mismatch, and type of linkage (ester or ether) on the rate of spontaneous intermembrane transfer. Our results suggest that the rate of spontaneous transfer of any phospholipid from a membrane lipid bilayer can be predicted on the basis of essentially independent contributions reflecting the nature of the bilayer environment, the lipid headgroup structure, and the length and degree of unsaturation of each acyl chain. While this general conclusion is similar to that recently advanced by

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[1] Abbreviations: apo A-I, apolipoprotein A-I; BHT, 2,6-di-*tert*-butylphenol; BMS-PE, *N*-[2-(*S*-bimanylthio)succinyl]phosphatidylethanolamine; (12-CA)-18-PC, 1-palmitoyl-2-[12-[[[7-(dimethylamino)-coumarin-4-yl]acetyl]methylamino]stearoyl]phosphatidylcholine; DABSyl, [4-[[4-(dimethylamino)phenyl]azo]phenyl]sulfonyl; (12-DABS)-18-PC, 1-palmitoyl-2-[12-(DABSylmethylamino)stearoyl]phosphatidylcholine; DMPC, 1,2-dimyristoyl-PC; DSPG, 1,2-distearoyl-PG; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; egg PC, PC from egg yolk; egg PE (PG), PE or PG prepared by transphosphatidylation from egg PC; HDL, high-density lipoprotein; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NBD, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl); *N*-oleoyl-CA-PE, 1-palmitoyl-2-[12-[[[7-(dimethylamino)coumarin-4-yl]acetyl]methylamino]stearoyl]-*N*-oleoylphosphatidylethanolamine; *N*-oleoyl-DABS-PE, 1-palmitoyl-2-[12-(DABSylmethylamino)stearoyl]-*N*-oleoylphosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SOPG, 1-stearoyl-2-oleoyl-PG; TLC, thin-layer chromatography; TNBS, sodium 2,4,6-trinitrobenzenesulfonate; Tris, tris(hydroxymethyl)aminomethane.

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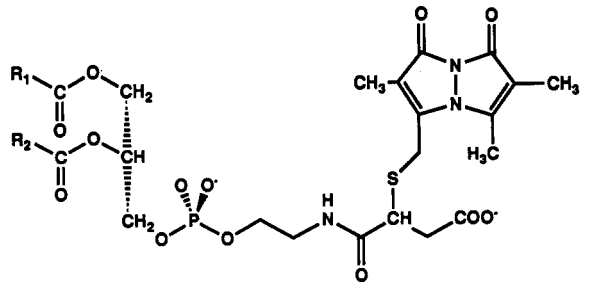

FIGURE 1: Structure of BMS-PE. The configuration of the mercapto-substituted carbon has not been assigned but is probably racemic.

Pownall *et al.* (1991) to predict the rates of transfer of different phospholipids in a model lipoprotein system, the magnitudes of the latter (acyl chain) contributions appear to be very different in the lipid bilayer *vs* model lipoprotein systems.

MATERIALS AND METHODS

Materials. Egg PC and synthetic homoacyl and lysophosphatidylcholines were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL); polyunsaturated PCs and lysolipids were stored at −80 °C and used for syntheses within a few days of receipt. Fatty acids and cholesterol were obtained from Nu-Chek Prep (Elysian, MN). Phospholipase D (from *Streptomyces sp.* AA 586) was a generous gift of Dr. Shigeyuki Imamura (Toyo Jozo Co., Tokyo, Japan). Distearoyl- and 1-stearoyl-2-oleoyl-PGs were synthesized from the corresponding PCs by phospholipase D-mediated transphosphatidylation in the presence of aqueous glycerol (Shuto *et al.*, 1987). (12-DABS)-18-PC and (12-CA)-18-PC, prepared as described previously (Silvius *et al.*, 1987), were transphosphatidylated with phospholipase D in the presence of ethanolamine (Juneja *et al.*, 1988), then acylated with oleic anhydride (8 h at 25 °C in 99:1 chloroform/triethylamine), and purified by preparative TLC to yield the corresponding *N*-oleoyl-PEs. [9,10(N)-^{3}H]Myristic acid and cholesteryl [1-^{14}C]oleate were obtained from Amersham Canada (Oakville, ON, Canada).

Mixed-acyl phosphatidylcholines were prepared as described previously (Mason *et al.*, 1981), including BHT (50 μg/mg fatty acid) for acylation reactions involving polyunsaturated fatty acids. PCs were purified by "flash" chromatography (Still *et al.*, 1978) on silica gel, eluting first with 80:20:0 and 75:25:0.5 $CHCl_3/CH_3OH$/water (discarded), and then with 65:35:1 $CHCl_3/CH_3OH$/water, pooling fractions found pure by TLC. PCs were converted successively to PEs, to *N*-(*S*-acetylthio)-PEs, and to BMS-PEs as described previously (Silvius & Zuckermann, 1993), performing all reactions under an inert atmosphere and with exclusion of light. All BMS-PEs prepared gave single sharp spots by TLC in 50:20:10:10:5 $CHCl_3$/acetone/$CH_3OH/CH_3COOH/H_2O$. The structure shown in Figure 1 was established by NMR spectral analysis of the *S*-acetylthio precursor, which revealed a clear NOESY peak between the methene and amide protons shown but no other detectable NOE couplings across the amide bond.

For polyunsaturated phospholipids all of the above reactions were carried out in the presence of 5 mg/mL BHT. BHT (50 μg/mL) was also included in all solvents used for purification of these species and was finally removed by repeatedly precipitating the final BMS-PEs from cold methanol with barium acetate (Comfurius & Zwaal, 1977). The purified polyunsaturated probes were finally combined with BHT (0.1 mol/mol of BMS-PE), stored at −80 °C, and handled under an inert atmosphere with essentially complete exclusion of light.

1-Acyl(1-alkyl)-2-([9,10-^{3}H]myristoyl)-PCs were synthesized by a microscale modification of the method of Mason *et al.* (1981), acylating the appropriate lyso-PCs with a 2-fold excess of tritiated myristic anhydride (specific activity 0.5 mCi/μmol). The labeled PCs were purified on microcolumns of silica gel 60 packed in Pasteur pipets, washing extensively with 95:5:0, 80:20:0.5, and 75:25:0.5 $CHCl_3/CH_3OH/H_2O$ (discarded) to remove fatty acid and catalyst and then eluting the labeled PC with 65:35:1 $CHCl_3/CH_3OH/H_2O$. Elution was monitored by TLC and scintillation counting.

Vesicle Preparation. Lipid samples, dried down from chloroform under nitrogen and further dried for several hours under high vacuum, were bath-sonicated to virtual clarity in 50 μM DTPA, pH 7.4, under nitrogen. One-ninth volume of 10-fold concentrated buffer (1.5 M KCl, 100 mM Tris or phosphate, 1 mM EDTA, 0.5 mM DTPA, pH 8.5) was added, and sonication was continued for 3 min. The samples were then repeatedly frozen in ethanol/dry ice and thawed (three times in most cases, eight times for dipentadecanoyl-PC vesicles). Egg PC/egg PG/egg PE (85:10:5) vesicles prepared by sonication/freeze-thawing in this manner gave encapsulated volumes [by the carboxyfluorescein-trapping assay of Wilschut *et al.* (1980)] of ca. 10 μL/μmol of lipid, corresponding to an apparent average diameter of 280 nm, and exposed 47–52% of their total PE on their outer surfaces as assessed by the TNBS reactivity assay of Nordlund *et al.* (1981). Vesicles prepared in this manner are referred to hereafter simply as "large unilamellar vesicles".

BMS-PEs were "asymmetrically" incorporated at 0.5 mol % into donor vesicles (Gardam *et al.*, 1988), by successively mixing the vesicles (1 mM lipid) at 37 °C with one-tenth volume of 10-fold concentrated buffer and nine-tenths volume of BMS-PE (5 μM) freshly sonicated into 50 μM DTPA, pH 8.5. The mixtures were incubated under nitrogen in the dark, normally for 90 min at 37 °C except for the following species: 16/16-BMS-PE (90 min, 43 °C); 16/18- and 18/16-BMS-PE (3 h, 43 °C); 18/18c^9- and 18c^9/18-BMS-PE (8 h, 37 °C); 18/(polyunsaturated)-BMS-PEs (5 h, 37 °C); and 18/18-BMS-PE (4 h, 48 °C followed by 12 h, 37 °C). Lipid extraction and TLC analysis of the donor vesicles after labeling as above revealed no evidence of lipid or probe degradation. As found previously (Gardam *et al.*, 1988; Silvius & Zuckermann, 1993), >95% of the probe molecules incorporated into donor vesicles by this procedure were readily available for transfer into subsequently added acceptor vesicles.

Assay Methods. Intervesicle transfer of BMS-PEs was assayed essentially as described previously (Silvius & Zuckermann, 1993) by monitoring the time-dependent enhancement of fluorescence accompanying transfer of BMS-PE molecules from donor vesicles containing 1.5 mol % *N*-oleoyl-DABS-PE to quencher-free acceptor vesicles. Control experiments using lipid vesicles labeled with 0.5 mol % *N*-oleoyl-DABS-PE and the nonexchangeable energy-transfer donor *N*-oleoyl-CA-PE demonstrated negligible transfer of the quencher to unlabeled acceptor vesicles (<2% transfer in 100 h) under the conditions of our experiments.

Donor and acceptor vesicles (normally 60 and 400 nmol, respectively, per 3-mL sample) were mixed at 37 °C in 150 mM KCl, 10 mM Tris, 0.5 mM EDTA, and 0.05 mM DTPA, pH 8.5 to initiate the probe-transfer process, either in the fluorimeter or, for longer incubations, in a separate vessel with exclusion of light and air (using nitrogen-purged buffers). Samples were withdrawn from these incubation mixtures at various times, quenched by cooling to 0 °C and subsequently rewarmed to 22 °C for measurement of fluorescence before and after addition of Triton X-100 to 1% (w/v). The

maximum possible dequenching due to probe transfer was determined using samples preincubated for long times (2–48 h at 37 °C, depending on the probe-transfer rate). TLC analysis of the lipids extracted from such incubation mixtures showed no evidence of degradation of the BMS-PEs or unlabeled lipids under these conditions.

Rate constants for intervesicle transfer of probe molecules were determined by fitting time courses of probe transfer to the first-order rate equation

$$F_N(t) = F_N^{max} - (F_N^{max} - F_N^0) \exp(-k_d t)$$

where $F_N(t)$, F_N^0, and F_N^{max} represent the fluorescence of the sample (normalized to the fluorescence measured after Triton addition) at time t, zero time, and "infinite" time, respectively. F_N^0 and F_N^{max} as well as k_d were normally left as adjustable parameters in the fitting procedure, but the values thereby estimated agreed very well with those measured for samples at zero time and after lengthy preincubation, respectively. For very slowly exchanging species (notably 18/18-BMS-PE), the value of F_N^{max} (which was essentially constant for different BMS-PEs in a given experiment) was estimated from data obtained for faster-exchanging probes in the same experiment and was incorporated as a fixed parameter in the fitting equation.

Intervesicle transfer of ^{3}H-labeled phosphatidylcholines was assayed with minor modifications of the method of Jones and Thompson (1989). Briefly, donor lipid vesicles, composed of 85:15 (mol/mol) egg PC/SOPG or egg PC/DSPG and labeled with trace amounts of tritiated PC, were incubated under nitrogen at 37 °C with egg PC acceptor vesicles (labeled with a trace of [^{14}C]cholesteryl oleate) in 25 mM KCl, 10 mM MOPS, 0.1 mM EDTA, and 50 μM DTPA, pH 7.0. At various times 50-μL aliquots of the incubation mixtures were applied to chilled minicolumns of DEAE-Sephacel (bed volume 0.5 mL) that were packed in 1-mL disposable syringes and prewashed with 300 nmol of sonicated egg PC vesicles followed by 2 mL of buffer before sample application. The columns were then eluted with 1.25 mL of ice-cold buffer, and the eluates were mixed with 12.5 mL of Cytoscint (ICN) and counted for ^{3}H and ^{14}C with appropriate spillover corrections. Recovery of acceptor vesicles was routinely ≥80% while retention of donor vesicles (assayed in parallel control runs) was >99.7%. Measured time courses of [^{3}H]phosphatidylcholine transfer were analyzed using a first-order rate equation as for the BMS-PE transfer processes discussed above. For all species save the slowly-transferring 18/14-PC the fraction of readily transferable PC (0.49–0.53) as well as the initial rate of transfer could be determined accurately by this approach; for the latter species the readily transferable fraction was set equal to the mean value measured for the other species examined.

RESULTS

Basic Properties of BMS-PEs. The general structure of BMS-PE is shown in Figure 1. The synthesis and some basic properties of this fluorescent lipid have been described previously (Silvius, 1993). Two properties of BMS-PEs make them particularly well suited to investigate the effects of acyl chain structure on the kinetics of phospholipid transfer between membranes. First, after initial dispersal in low-ionic strength media these probes rapidly and quantitatively insert into the outer monolayers of lipid vesicles. The insertion process [which is readily monitored by fluorescence enhancement (Silvius & Zuckermann, 1993)] occurs very efficiently for BMS-PEs of a variety of acyl chain lengths, with half-times at 37 °C and pH 8.5 ranging from <2 s for 12/12-BMS-PE to ca. 8 min for species such as 18c^9/18c^9-BMS-PE. Replicate portions of a single preparation of donor vesicles can thus be labeled in the outer monolayer with various BMS-PEs, maximizing the fraction of probe readily available for intervesicle transfer and permitting particularly well-controlled comparisons of the interbilayer transfer rates for different probe species.

Comparisons of the emission spectral maxima for BMS-PEs in various organic solvents, water/solvent mixtures, and egg PC vesicles indicated that in the latter system the bimane group senses a local dielectric constant of approximately 60 (data not shown). This relatively high value suggests that in lipid bilayers the bimane group of BMS-PE is disposed more toward the aqueous phase than toward the interfacial or hydrophobic regions.

As described previously (Silvius & Zuckermann, 1993), the fluorescence of BMS-PE probes and their derivatives is efficiently quenched by low levels of DABSyl-labeled lipids present in the same bilayer (Silvius *et al.*, 1987). This property can be used to monitor accurately and conveniently the kinetics of intervesicle transfer of fluorescent probes (Nichols & Pagano, 1982; Silvius & Zuckermann, 1993), simply by monitoring the time-dependent enhancement of fluorescence after unlabeled acceptor vesicles are added to donor vesicles labeled with both the fluorescent probe and a nonexchangeable fluorescence quencher (here, *N*-oleoyl-DABS-PE). All experiments described in this paper used large unilamellar vesicles prepared as described in Materials and Methods. In preliminary experiments we established that under the experimental conditions used here the kinetics of intervesicle probe transfer were exponential, independent of the mole fraction of BMS-PE in the donor vesicles (from 0.1 mol % to at least 1 mol %), and independent of the total vesicle concentration (at a fixed donor/acceptor ratio) up to at least 4 mM lipid. These results, which were established for all donor/acceptor systems examined using a representative variety of BMS-PE species, indicate that intervesicle probe transfer occurs via diffusion of individual probe molecules through the aqueous phase and does not require intervesicle collisions (Nichols & Pagano, 1982; Jones & Thompson, 1989). Under these conditions, the rate constant measured for intervesicle transfer of BMS-PE molecules can be equated to k_d, the first-order rate constant for desorption of probes from the vesicle surface (Roseman & Thompson, 1980; Nichols & Pagano, 1982).

The value of k_d for several BMS-PE species in egg PC vesicles was found to vary with pH, with an apparent pK_a of ca. 5.2–5.5 (not shown), presumably reflecting the titration of the BMS-PE carboxyl group. The intervesicle transfer of BMS-PEs was therefore measured at pH 8.5, where the pH dependence of k_d was weak even for donor vesicles containing anionic lipids.

Effects of Lipid Environment. The desorption rates for several representative BMS-PE species, differing in acyl chain length and in degree of unsaturation, were monitored as a function of the vesicle lipid environment. These experiments employed probes whose transfer kinetics could be monitored continuously in the spectrofluorimeter over relatively short time periods.

As shown in Figure 2, for the homologous series 12/12- to 15/15-BMS-PE the rate constant for probe desorption from several different types of lipid vesicles declines exponentially with increasing acyl chain length. From the slopes of these

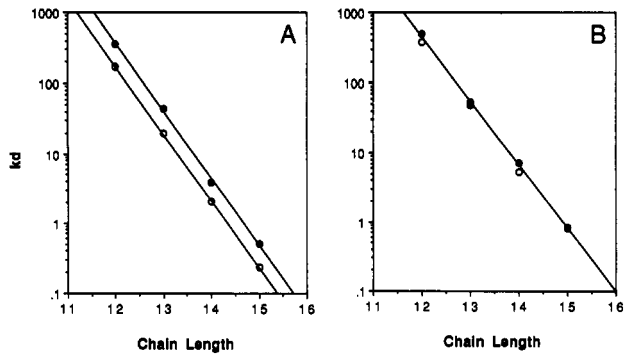

FIGURE 2: First-order rate constants k_d (in h^{-1}) measured at 37 °C for desorption of homoacyl BMS-PEs with chains of the indicated carbon numbers from large unilamellar lipid vesicles. The lines shown are least-squares fits to the equation $(\ln k_d) = \ln(\kappa kT/h) - (\Delta G_d^{\ddagger}/RT) = C_1 + C_2(N_C)$, where C_1 and C_2 are fitting constants, N_C is the length of each acyl chain, and $\Delta G_d^{\ddagger}$ is the free energy of activation for desorption. The quantity $-(RTC_2)$ represents the incremental increase in $\Delta G_d^{\ddagger}$ when one methylene residue is added to each acyl chain of a saturated homoacyl BMS-PE. (A) Donor vesicles composed of (●) egg PC or (○) dipentadecanoyl-PC. (B) Donor vesicles composed of (●) 90:10 egg PC/egg PG or (○) 54:6:40 egg PC/egg PG/cholesterol. The regression line obtained for the cholesterol-containing vesicles is omitted for clarity. In this and succeeding figures the sizes of the data points are comparable to or greater than the relevant error estimates for the data points (see footnote 3).

plots, using absolute reaction-rate theory[2] (Glasstone *et al.*, 1941), we can estimate the incremental contribution of each added pair of methylene residues to the free energy of activation for BMS-PE desorption, $\Delta G_d^{\ddagger}$. The magnitude of this increment is very similar for probe desorption from vesicles of unsaturated egg PC *vs* saturated dipentadecanoyl PC (1.36 ± 0.02 *vs* 1.35 ± 0.03 kcal mol^{-1}, respectively[3]), and for probe desorption from vesicles of 90:10 egg PC/egg PG without or with 40 mol % cholesterol (1.27 ± 0.03 *vs* 1.29 ± 0.03 kcal mol^{-1}, respectively).

In the experiments summarized in Figure 2A we consistently observed roughly 2-fold lower k_d values for desorption of BMS-PEs from saturated dipentadecanoyl-PC vesicles than from egg PC donor vesicles. A very similar result was observed using vesicles prepared by extruding unsonicated lipids through 0.1-μm polycarbonate filters (MacDonald *et al.*, 1991), although the absolute k_d values obtained using such vesicles were uniformly some 2-fold higher than those measured using sonicated/freeze-thawed vesicles. These results suggest that vesicle composition, as well as vesicle curvature (Jones & Thompson, 1990; Wimley & Thompson, 1990), may influence to some degree the absolute value of k_d measured for any given phospholipid species. Importantly, however, as already noted, the *relative* rates of desorption of different BMS-PE species from large unilamellar lipid vesicles appear to be virtually independent of the vesicle composition.

The effects of different lipid environments on the desorption of unsaturated BMS-PEs were also examined using the above donor–vesicle systems. 14/14c[9]-BMS-PE desorbed from large unilamellar egg PC and dipentadecanoyl-PC vesicles 6.7- and 6.8-fold faster, respectively, than did 14/14-BMS-PE, indicating that in these systems the introduction of one *cis* double bond decreases the value of $\Delta G_d^{\ddagger}$ by essentially the same amount (1.17–1.18 kcal mol^{-1}). A similar comparison of the desorption of these probes from 90:10 egg PC/egg PG vesicles without or with 40 mol % cholesterol indicated that in these cases the introduction of one *cis* double bond lowers $\Delta G_d^{\ddagger}$ by 0.96 and 1.12 kcal mol^{-1}, respectively. In parallel experiments comparing the desorption kinetics of 16c[9]/16c[9]- *vs* 16/16-BMS-PE, it was observed that the introduction of one double bond per acyl chain decreased the value of $\Delta G_d^{\ddagger}$ by 2.23, 2.26, 2.16, and 2.24 kcal mol^{-1} for donor vesicles composed of egg PC, dipentadecanoyl-PC, 90:10 egg PC/egg PG, and 54:6:40 egg PC/egg PG/cholesterol, respectively. These decreases in $\Delta G_d^{\ddagger}$ are almost precisely double those measured in the same systems comparing 14/14c[9]- to 14/14-BMS-PE.

In parallel experiments we found that BMS-PE probes "symmetrically" incorporated into donor vesicles (by including the BMS-PE probe directly in the lipid mixture used for donor preparation) gave k_d values very similar to those estimated above using the same probes "asymmetrically" incorporated into the donor vesicles. However, the fraction of BMS-PE readily available for transfer from symmetrically labeled donor vesicles was almost exactly one-half that measured using asymmetrically labeled donors (data not shown).

Effects of Acyl Chain Length and Unsaturation. To explore more widely the effects of BMS-PE acyl chain composition on the rate of desorption from the bilayer, we next measured the rates of desorption of a variety of BMS-PE species from 90:10 egg PC/egg PG vesicles at 37 °C, giving the results summarized in Table I and in Figures 3 and 4. For shorter-chain BMS-PEs, at least the initial portion of the probe-transfer process could be monitored continuously in the fluorimeter. Mixtures containing longer-chain BMS-PEs were incubated

[2] Using absolute reaction-rate theory, enthalpies and free energies of activation are estimated from the equations

$$\Delta G_d^{\ddagger} = \ln(\kappa kT/h) - \ln(k_d) \quad (1)$$

where the parameter κ is normally set equal to 1, and

$$\Delta H_d^{\ddagger} = -R[\mathrm{d}(\ln k_d)/\mathrm{d}(1/T)] - RT \quad (2)$$

Alternate estimates of the enthalpy and free energy of activation for lipid desorption can be obtained using the approach of Aniansson *et al.* (1976), as suggested by Nichols (1985). This theoretical treatment leads to the equation

$$k_d = [D_m/(l_b)^2] \exp(-\Delta G_d^{\ddagger}/RT) \quad (3)$$

where D_m is the effective diffusion coefficient of the molecule as it traverses the region of the free-energy surface that lies within RT units of the free energy of the transition state for desorption, and l_b is the spatial extent of this region perpendicular to the bilayer surface. Substituting D_m by (kT/f) in the above equation and rearranging, we obtain the equation

$$\Delta H_d^{\ddagger} = -R[\mathrm{d}(\ln k_d)/\mathrm{d}(1/T)] - RT - R[\mathrm{d}(\ln \eta)/\mathrm{d}(1/T)] \quad (4)$$

where the last term arises from the linear dependence of the frictional coefficient f on the medium viscosity η (normally set equal to the viscosity of bulk water). Using appropriate estimates for D_m and l_b [see Nichols (1985)], when applied to experimental rate–temperature profiles these equations lead to values for $\Delta G_d^{\ddagger}$ (at 37 °C), $\Delta H_d^{\ddagger}$, and $\Delta S_d^{\ddagger}$ that are roughly 2.6 kcal mol^{-1}, 3.9 kcal mol^{-1}, and 4.2 eu less, respectively, than those estimated using absolute reaction-rate theory. However, the two theories provide identical estimates of the *differences* in the activation parameters between different species compared under the same conditions.

[3] Three major sources of variability influence the values reported in this study (normally determined from triplicate runs in triplicate experiments). As discussed before (Silvius & Zuckermann, 1993), while the measured value of k_d for a given probe may vary by up to 30% between experiments (possibly reflecting variations in factors such as average vesicle dimensions), the *ratios* of the k_d values for desorption of different probes from the same population of donor vesicles typically vary by <10% between experiments. Estimated errors from these sources are not indicated at each point in the text but in all cases are less than these limits: for absolute k_d values, <±20%; for ratios of k_d values for different probes or different temperatures, <±8%; for absolute values of $\Delta G_d^{\ddagger}$, <±110 cal mol^{-1}; and for differences in $\Delta G_d^{\ddagger}$ between different species, <±50 cal mol^{-1}. A third source of "error" arises in estimates of parameters based on regression analyses of the k_d values for multiple lipid species. For such analyses, where the major source of "error" is systematic variations in the k_d values of individual probe species not accounted for by the regression parameters, estimated uncertainties for these parameters are indicated in the text.

Table I: Rate Constants Measured for Desorption of BMS-PEs from Large Unilamellar 90:10 Egg PC/Egg PG Vesicles at 37 °C[a]

species	k_d, h^{-1}	$t_{1/2}$, h[b]
12/12	2.18×10^{2}	3.18×10^{-3}
13/13	2.07×10^{1}	3.35×10^{-2}
12/14	1.60×10^{1}	4.33×10^{-2}
12/14c^9	8.38×10^{1}	8.27×10^{-3}
14/14	2.95×10^{0}	2.35×10^{-1}
14/14c^9	1.55×10^{1}	4.47×10^{-2}
15/15	3.96×10^{-1}	1.75×10^{0}
14/16	3.38×10^{-1}	2.05×10^{0}
16/14	3.35×10^{-1}	2.07×10^{0}
14/16c^9	2.11×10^{0}	3.28×10^{-1}
16/14c^9	1.70×10^{0}	4.08×10^{-1}
16/16	4.96×10^{-2}	1.40×10^{1}
14/18	3.85×10^{-2}	1.80×10^{1}
18/14	6.06×10^{-2}	1.14×10^{1}
16/16c^9	2.51×10^{-1}	2.76×10^{0}
14/18c^9	2.71×10^{-1}	2.56×10^{0}
18/14c^9	2.49×10^{-1}	2.78×10^{0}
18c^9/14	2.91×10^{-1}	2.38×10^{0}
16c^9/16c^9	1.31×10^{0}	5.27×10^{-1}
16/18	7.64×10^{-3}	9.07×10^{1}
18/16	7.46×10^{-3}	9.29×10^{1}
16/18c^9	4.52×10^{-2}	1.53×10^{1}
18/16c^9	4.87×10^{-2}	1.42×10^{1}
18c^9/16	4.73×10^{-2}	1.47×10^{1}
18/18	1.87×10^{-3}	3.71×10^{2}
18/18c^9	9.12×10^{-3}	7.60×10^{1}
18c^9/18	8.84×10^{-3}	7.84×10^{1}
18c^9/18c^9	4.16×10^{-2}	1.67×10^{1}

[a] Triplicate determinations of k_d in each of three experiments were averaged, and the resulting mean values from each experiment were than geometrically averaged to give the values tabulated. The maximal estimated uncertainty for k_d is ±20% of the value given in all cases. However, the maximum uncertainty in the *ratio* of any two tabulated k_d values is ±8% (see footnote 3 for further details). [b] Estimated half-times for spontaneous interbilayer exchange of the BMS-PEs in this experimental system, calculated from the equation $t_{1/2} = (\ln 2)/k_d$.

outside the fluorimeter with exclusion of light and air and sampled at various times to monitor the time course of probe transfer. For probes of intermediate chain length k_d was determined by both methods, with very good agreement in all cases.

In Figure 3 the desorption rate constants measured for the BMS-PEs listed in Table I are plotted *vs* their total carbon number. In this semilog presentation it can be seen that the data for saturated, unsaturated, and diunsaturated BMS-PEs, respectively, cluster along three nearly parallel lines. From the slopes and vertical displacements of the fitted lines we calculate that each addition of a methylene group increases ΔG_d^* by 605 ± 15 cal mol^{-1} for saturated species and by similar amounts (570 ± 10 cal mol^{-1} and 530 ± 30 cal mol^{-1}, respectively) for mono- and diunsaturated species. The addition of a *cis* double bond to one or to both acyl chains (at a constant total carbon number) decreases ΔG_d^* on average by 1020 or 1980 cal mol^{-1}, respectively.

In separate experiments we observed that the introduction of a 9-*cis* or 9-*trans* double bond into the 16-carbon chain of 14/16-BMS-PE increased the value of k_d by 4.9- or 3.25-fold, corresponding to a reduction of ΔG_d^* by 975 cal mol^{-1} or by 725 cal mol^{-1}, respectively (data not shown). The effect of a *trans*-unsaturated chain on the rate of probe desorption is thus clearly more similar to that of a *cis* unsaturated chain than to that of a saturated chain of equal carbon number.

It can be seen from Table I and Figure 3 that different saturated BMS-PEs with the same total carbon number show quite similar values for k_d (and hence for ΔG_d^*), with only small variations depending on the relative lengths of the two

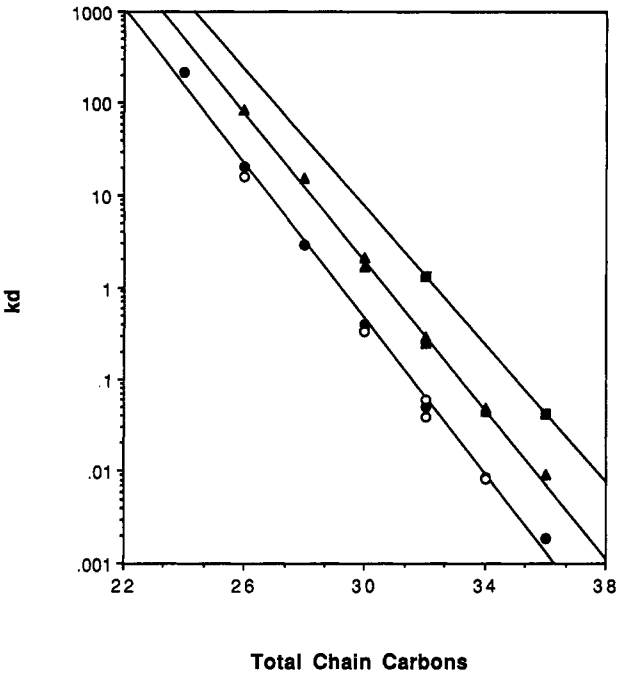

FIGURE 3: Measured k_d values (h^{-1}) for the desorption of BMS-PEs of the indicated summed acyl chain lengths from 90:10 egg PC/egg PG large unilamellar vesicles at 37 °C. The data plotted are those given in Table I. (●) Saturated homoacyl BMS-PEs; (○) saturated heteroacyl BMS-PEs; (▲) saturated/monounsaturated heteroacyl BMS-PEs; (■) di(monounsaturated) homoacyl BMS-PEs.

chains. The same is true for different monounsaturated BMS-PEs with a constant total carbon number but different positions and relative lengths of the saturated and unsaturated acyl chains. Within most of these sets of isomeric BMS-PEs the value of ΔG_d^* varies by no more than 100–150 cal mol^{-1}, less than one-fourth of the incremental change in ΔG_d^* upon addition of a single methylene residue to one acyl chain (the sole exception is the set 16/16-, 14/18-, and 18/14-BMS-PE, where the range of ΔG_d^* values is 280 cal mol^{-1}). Selective 2-position deacylation of the parent heteroacyl phosphatidylcholines with phospholipase A_2, followed by analysis of the liberated fatty acid and lysophosphatidylcholine products (Kates, 1986), confirmed that these species were in fact acylated with high positional specificity (>90% in all cases), in agreement with our previous results using this method of acylation [see Abrams *et al.* (1992)].

The above experiments were also extended to examine the desorption of several polyunsaturated BMS-PEs from egg PC/egg PG vesicles. Because of the greater potential sensitivity of these BMS-PE species to (photo)oxidation, additional precautions were taken to minimize their exposure to light and air (see Materials and Methods), and the donor and acceptor vesicles included 0.5 mol % BHT as an antioxidant. In control experiments this level of BHT had no significant effect on the rate of intervesicle transfer of several saturated and monounsaturated BMS-PEs.

The desorption rate constants measured for three series of MBS-PEs with a constant total carbon number and increasing degrees of unsaturation are plotted in Figure 4. Most of the probes examined fall into three families, comprising C_{16}/C_{18}(unsaturated), C_{18}/C_{18}(unsaturated), and di-C_{18}(unsaturated) species, which define the three curves shown in Figure 4. While within each family the value of k_d rises steadily with increasing number of double bonds, the contribution of each successive double bond to this enhancement of the desorption rate is not constant. The introduction of the first double bond to one or both acyl chains produces the greatest

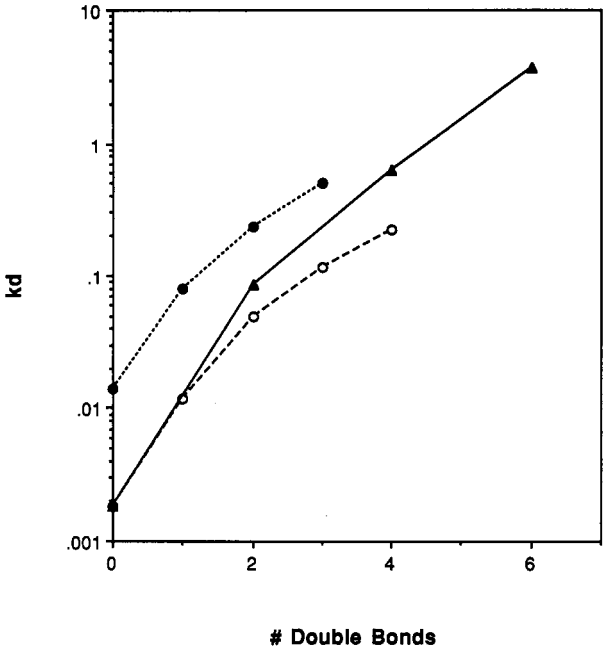

FIGURE 4: Measured rate constants k_d (h^{-1}) for desorption of BMS-PEs of fixed total carbon number and varying degrees of unsaturation from 90:10 egg PC/egg PG large unilamellar vesicles at 37 °C. (●) Data for 16/18-, 16/18c^9-, 16/18cc^{9,12}-, and 16/18ccc^{9,12,15}-BMS-PE. (▲) Data for 18/18-, di-18c^9-, di-18:2cc^{9,12}-, and di-18:3ccc^{9,12,15}-BMS-PE. (○) Data for 18/18-, 18/18c^9-, 18/18cc^{9,12}-, and 18/18ccc^{9,12,15}-BMS-PEs and 16/20:4cccc^{5,8,11,14}-BMS-PE. Data points shown are averages over three experiments, calculated as described for the data in Table I. The calculated decrements in the value of ΔG_d^* with addition of successive double bonds within these series are as follows: (●) −1080, −655, and −460 cal mol^{-1}; (▲) −2325, −1230, and −1100 cal mol^{-1} (for addition of successive *pairs* of double bonds); (○) −1090, −890, −530, and −400 cal mol^{-1}. The value of k_d measured for 18/20:4cccc^{5,8,11,14}-BMS-PE in the same experiments (not plotted) was 3.93×10^{-2} h^{-1}, corresponding to a ΔG_d^* value 1070 cal mol^{-1} higher than that for 16/20:4cccc^{5,8,11,14}-BMS-PE.

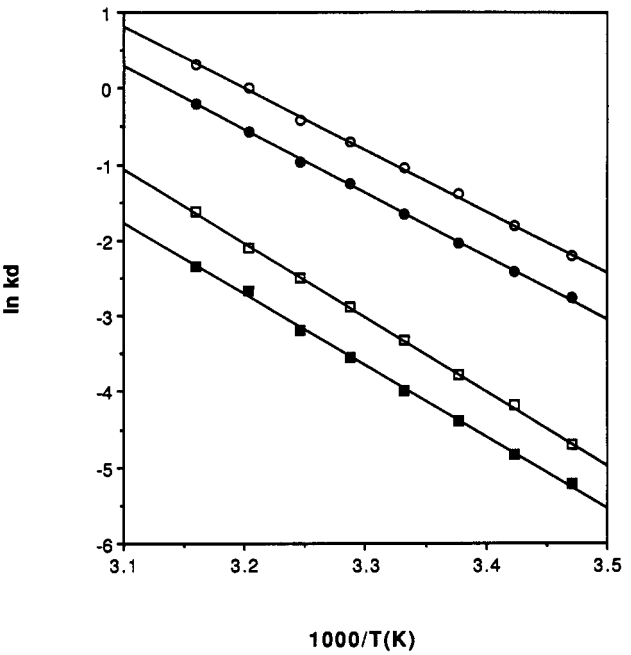

FIGURE 5: Arrhenius plots for the transfer of (○) 13/13-, (●) 14/14c^9-, (□) 14/14-, and (■) di-16c^9-BMS-PE between 90:10 egg PC/egg PG large unilamellar vesicles. Estimates of ΔH_d^* values, obtained from the fitted least-squares lines as described in footnote 2, are given in the text.

decrease in ΔG_d^* (ca. 1100 cal mol^{-1}), while addition of further double bonds *to the same chain(s)* reduces ΔG_d^* by progressively smaller increments (e.g., some 500 cal mol^{-1} for the addition of a third double bond to a 9,12-diunsaturated chain). Interestingly, these effects seem to operate essentially independently for the two acyl chains.

The temperature dependence of k_d was examined for the transfer of four representative BMS-PEs from 90:10 egg PC/egg PG vesicles, as illustrated in Figure 5. The measured enthalpies of activation (ΔH_d^*) for desorption of 13/13-, 14/14-, 14/14c^9-, and 16c^9/16c^9-BMS-PE from these vesicles, estimated using absolute reaction-rate theory (Glasstone *et al.*, 1941),[2] were 15.5 ± 0.2, 18.8 ± 0.1, 16.0 ± 0.2, and 18.3 ± 0.3 kcal mol^{-1}, respectively. It is apparent that the value of ΔH_d^* increases more steeply with increasing chain length, and decreases more rapidly with increasing unsaturation, than does the value of ΔG_d^* at 37 °C (estimated in these experiments to increase by 1.28 kcal mol^{-1} for 14/14- *vs* 13/13-BMS-PE, and to decrease by 940 cal mol^{-1} for 14/14c^9- *vs* 14/14-BMS-PE). It thus appears that the observed variation in ΔG_d^* with acyl chain length and unsaturation for the BMS-PEs is driven by a parallel but still larger variation in ΔH_d^*, which is partially offset by a parallel variation in the entropy of activation.

Comparison of BMS-PE and [^{3}H]PC Desorption Rates. In a final series of experiments, we compared the desorption rates of a series of 1-acyl-2-myristoylphosphatidylcholines and BMS-PEs from large unilamellar PC/PG vesicles. The transfer of [^{3}H]myristate-labeled PCs from these donor vesicles to large unilamellar egg PC acceptor vesicles was assayed as described in Materials and Methods, using ion-exchange chromatography to separate donor and acceptor vesicles after coincubation for various times (McLean & Phillips, 1981; Jones & Thompson, 1989, 1990). Under the standard conditions of these experiments (large unilamellar vesicles, 4 mM total lipid), the rate constants measured for [^{3}H]PC transfer were essentially identical to those determined by extrapolating to zero lipid concentration, in agreement with previous results for similar systems (Jones & Thompson, 1990; Wimley & Thompson, 1991).

The rate constants estimated for desorption of a series of 1-acyl-2-[^{3}H]myristoyl-PCs from 85:15 egg PC/distearoyl PG (DSPG) vesicles at 37 °C are summarized in Figure 6 (open circles). The value of k_d measured for dimyristoyl-PC (DMPC) in this system (0.66 h^{-1}) is roughly 2-fold greater than the value estimated by Wimley and Thompson (1990) for desorption of this species from large unilamellar DMPC vesicles. This difference compares well to the roughly 2-fold difference observed here between the rates of desorption of BMS-PEs from vesicles composed of egg PC *vs* dipentadecanoyl-PC (Figure 2A). The estimated mean increment in ΔG_d^* upon elongation of the 1-position chain by two methylene groups is 1385 ± 60 cal mol^{-1}, a value that is fairly constant with increasing chain length. Addition of a 9′-*cis* double bond to the 1-position chain of 1-stearoyl-2-myristoyl-PC reduces ΔG_d^* by an estimated 1385 cal mol^{-1} (k_d increases by 9.5-fold), while replacement of the 1-position ester by an ether linkage in 1-palmitoyl-2-myristoyl-PC decreases k_d by 2.6-fold, corresponding to an increase in ΔG_d^* of ca. 600 cal mol^{-1} (data not shown).

In a parallel series of experiments we measured the transfer of a series of 1-acyl-2-myristoyl-BMS-PEs between large unilamellar donor and acceptor vesicles of the same compositions as those used above to measure [^{3}H]PC transfer (Figure 6, filled triangles). While the absolute rates of desorption measured for the BMS-PEs in this system exceed those for the corresponding PCs by roughly 5-fold (mean value 4.92-fold, range 4.2- to 5.8-fold for five PC/BMS-PE pairs compared), the pattern of variation of k_d with the structure

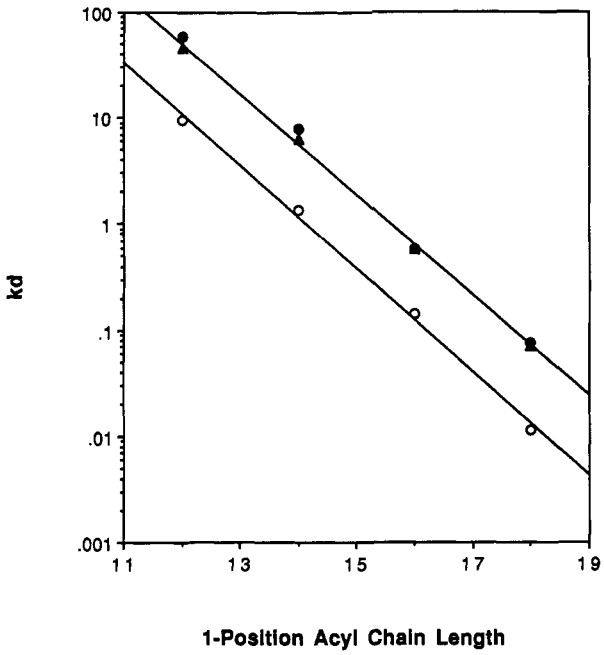

FIGURE 6: Measured k_d values for the desorption of saturated 1-acyl-2-myristoyl phospholipids from PC/PG large unilamellar vesicles at 37 °C. (○) Desorption of PCs from 85:15 egg PC/DSPG vesicles; (▲) desorption of BMS-PEs from 85:15 egg PC/DSPG vesicles; (●) desorption of BMS-PEs from 85:15 PC/SOPG vesicles. The slopes of the best-fit least-squares lines (omitted for clarity for the last data set) correspond to increments in ΔG_d^* of 1385 ± 55, 1345 ± 35, and 1390 ± 50 cal mol^{-1}, respectively.

of the 1-position acyl chain is very similar for the two types of lipids. The average increments in ΔG_d^* accompanying addition of a pair of methylene groups or a *cis* double bond to the 1-acyl chain in this series of BMS-PEs (1345 ± 35 and −1355 ± 40 cal mol^{-1}, respectively) very closely match the analogous values measured for the corresponding series of PCs (see above). These results are consistent with previous suggestions (Nichols & Pagano, 1982; Massey *et al.*, 1982a; Gardam *et al.*, 1988; Shin *et al.*, 1991; Pownall *et al.*, 1991; Slater *et al.*, 1993) that the phospholipid headgroup and the acyl chains contribute essentially independently to determine the value of ΔG_d^* for a particular lipid species.

In the experiments described in this section, DSPG was used as the anionic lipid in the donor vesicles to minimize the potential for PG transfer to the acceptor vesicles (which could impair their separation from donor vesicles). In parallel experiments, k_d values were also measured for desorption of the various BMS-PEs from donor vesicles containing 15 mol % 1-stearoyl-2-oleoyl-PG in place of DSPG, giving very similar results (Figure 6, filled circles).

DISCUSSION

Fluorescent-labeled phospholipids have previously been used in numerous studies of the mechanism and energetics of phospholipid transfer between bilayers, in large part because of the simplicity and accuracy with which their intervesicle transfer can be monitored (Roseman & Thompson, 1980; Nichols & Pagano, 1981, 1982; Frank *et al.*, 1983; Massey *et al.*, 1984; Nichols, 1985; Nichols, 1986; Homan & Pownall, 1988; Jones & Thompson, 1989, 1990; Slater *et al.*, 1993; Silvius & Zuckermann, 1993). However, the probes examined in most of these studies carry their fluorescent group on an acyl chain, a potential complicating factor in efforts to use such probes to explore the normal effects of phospholipid acyl chain structure (and packing within the bilayer) on interbilayer transfer kinetics. These concerns are mitigated for BMS-PEs by the basic structure of these probes and by the apparent disposition of the bimane group in a high-polarity environment in lipid bilayers, and in fact we find that PCs and BMS-PEs show almost identical patterns of variation of ΔG_d^* with the addition of methylene groups, or of a *cis* double bond, to their acyl chains. Interestingly, we have observed that synthetic NBD-PEs, whose fluorescent group appears to associate more strongly with the interfacial region of the bilayer (Chattopadhyay & London, 1987, 1988) desorb from egg PC/egg PG vesicles roughly 200 times more slowly than do BMS-PEs with the same acyl chains.

In general, the results presented here concerning the effects of acyl chain length on the rate of phospholipid desorption from lipid vesicles agree well with those reported previously using other experimental systems. Our estimates for the increase in ΔG_d^* per pair of methylene groups added to a homoacyl BMS-PE (1210–1360 cal mol^{-1} for the different systems examined) are very similar to those estimated by Ferrell *et al.* (1985) from measurements of transfer of saturated homoacyl PCs from small unilamellar vesicles of these species to erythrocyte membranes (ca. 1300 cal mol^{-1}). These estimates are also very similar to those reported by Nichols (1985) and by Homan and Pownall (1988) based on measurements of the desorption of 1-acyl-2-[6-(NBDamino)-hexanoyl]-PCs and of 1-acyl-2-(9-pyrenylnonanoyl)-PCs, respectively, from small unilamellar phosphatidylcholine vesicles. The increase that we observe in the value of ΔH_d^* for 14/14- over 13/13-BMS-PE (3.3 ± 0.3 kcal mol^{-1}) is also quite comparable to the increments in ΔH_d^* observed by Nichols (1985) between 1-acyl-2-[6-(NBDamino)hexanoyl]-PCs differing in total chain length by two methylene units. While absolute rates of phospholipid desorption can vary significantly with variations in bilayer composition and curvature [this study and Jones and Thompson (1989), Wimley and Thompson (1990), and Slater *et al.* (1993)], the *relative* rates of exchange observed for different lipids with a common basic structure but varying acyl chain length appear remarkably consistent, independent of both the details of the lipid (probe) structure and the nature of the bilayer environment. It should thus be possible to use data like those reported here to predict fairly quantitatively the relative rates of spontaneous desorption of various phospholipids, differing in their acyl chain moieties and/or hydrocarbon chain linkages, even for bilayer membranes of different composition from those examined here.

The tendency of acyl chain unsaturation to accelerate the spontaneous interbilayer transfer of phospholipids has not previously been explored in detail, although earlier studies have reported that *cis*-unsaturated lipid probes desorb faster than do saturated species of equal chain length from natural and model serum lipoproteins (Massey *et al.*, 1982b, 1984; Pownall *et al.*, 1991). Two properties of unsaturated phospholipids seem particularly likely to contribute to this effect. The first is the potential of the polarizable double bond(s) to enhance interactions between the lipid acyl chains and water in the transition state leading to desorption, in which the acyl chains of the desorbing molecule are thought to be largely displaced into the polar region adjacent to the bilayer (Aniansson *et al.*, 1976; Nichols, 1985; Jones & Thompson, 1990). The second is a generalized (modest) tendency of unsaturation to weaken interactions between the acyl chains of adjacent lipids in bilayers, which again could facilitate the displacement of a lipid's acyl chains from the bilayer interior. This second effect of lipid unsaturation is difficult to define, let alone to quantify, using any single experimental parameter. Nonetheless, on the basis of such quantities as gel-to-liquid

crystalline transition temperatures, monolayer molecular areas, and chain order parameters measured for various phosphatidylcholines, we can state that in general these effects are considerably more pronounced for *cis*- than for *trans*-unsaturated species (Seelig & Seelig, 1977; Seelig & Waespe-Sarcevic, 1978; Silvius, 1982; Siminovitch *et al.*, 1987) and that the introduction of the first *cis* double bond into a saturated phospholipid produces a greater incremental change in these quantities than do further stepwise additions of *cis* double bonds to either acyl chain (Demel *et al.*, 1967, 1972; Evans & Tinoco, 1978; Stubbs *et al.*, 1981; Coolbear *et al.*, 1983; Paddy *et al.*, 1985; Litman *et al.*, 1991). These trends are clearly reflected in the observed relative effects of *cis- vs trans*-unsaturation, and of increasing degrees of chain unsaturation, on the values of $\Delta G_d^{\ddagger}$ for different BMS-PEs of a fixed carbon number. Given the effects of chain unsaturation on some of the other phospholipid properties discussed above, we might in fact expect *cis- vs trans*-double bonds, or successive double bonds added to the same acyl chain, to produce even more strongly varying effects on the value of $\Delta G_d^{\ddagger}$ than we actually observe. We suggest that both the intrinsic polarizability of double bonds and their (position- and configuration-dependent) effects on lipid packing contribute significantly in reducing the values of $\Delta G_d^{\ddagger}$ for unsaturated lipids.

While the value of $\Delta G_d^{\ddagger}$ for a phospholipid depends strongly on the total carbon number and degree of unsaturation of the acyl chains, it appears that this parameter is only weakly influenced by the relative lengths of the two chains, or by the position of the unsaturated chain, for different BMS-PEs of a given total carbon number and degree of unsaturation. Some previous studies [discussed in Tanford (1980) and Nichols (1985)] have suggested that for double-chain amphiphiles the standard free energy change for the monomer–micellar equilibrium may depend strongly on the degree of mismatch in length between the two hydrocarbon chains, as well as on the total chain length. Since a lipid traversing the transition state for desorption from a bilayer appears to have acquired much of the character of the final monomeric state (Aniansson *et al.*, 1976; Nichols, 1985; Jones & Thompson, 1990), we might anticipate that the values of $\Delta G_d^{\ddagger}$ for different diacyl phospholipids would likewise depend substantially on the relative lengths and degrees of unsaturation of the 1- and 2-position acyl chains, even when the total numbers of carbons and double bonds for the two chains are held constant. By similar reasoning, we might predict that the incremental changes in $\Delta G_d^{\ddagger}$ accompanying addition of methylene residues or double bonds to one chain of a phospholipid should depend substantially on the relative lengths and positions of the acyl chains. In general, however, the results obtained here (and for the other systems discussed above) indicate that such effects are at best very modest. A plausible interpretation is that in the transition state for the desorption process the two acyl chains of a phospholipid interact only weakly, at least in their distal portions. An alternative (though in our opinion less likely) possibility is that in the above experimental systems acyl chain mismatch contributes quantitatively very similar increments to the net free energy of the lipid probe in the bilayer and in the transition state leading to desorption.

As already noted, several previous studies have examined the effects of varying acyl chain structure on the transfer of fluorescent (acyl chain-labeled) and, more recently, radiolabeled PCs between natural and model serum lipoproteins (Massey *et al.*, 1982a,b, 1984; Pownall *et al.*, 1991). In some respects the results obtained with these latter systems agree qualitatively with those observed here and in previous studies of lipid desorption from bilayer vesicles. Thus, the rates of phospholipid desorption from model or natural lipoproteins also decrease exponentially with increasing chain length, and the stepwise addition of two double bonds to a phospholipid progressively increases the desorption rate in substantial increments.

Noting the general similarities in the processes of phospholipid desorption from lipoproteins and bilayer vesicles, Pownall *et al.* (1991) have proposed a model for predicting the rates of desorption of different lipids from various lipidic structures, including bilayer vesicles, based on measurements of the transfer of radiolabeled PCs from recombinant high-density lipoprotein. Our results agree with one key assumption of this model, namely, that the two acyl chains of a phospholipid, as well as the polar headgroup, contribute essentially independently in determining the value of $\Delta G_d^{\ddagger}$ for desorption from a lipid aggregate. However, our findings do not support a second assumption of this model, namely, that the rate of desorption of a phospholipid from an extended bilayer can be predicted from its rate of desorption in the above model lipoprotein system, simply by applying a linear scaling constant to account for differences in the surface "qualities" of the two systems. In fact, the addition of methylene groups or double bonds to a phospholipid alters the kinetics of desorption much more strongly in the present lipid vesicle system (where $\Delta G_d^{\ddagger}$ increases by ca. 0.65 and −1.1 kcal mol^{-1}, respectively, per added methylene residue or *cis* double bond) than in the recombinant HDL system investigated by Pownall *et al.* (where the corresponding increments in $\Delta G_d^{\ddagger}$ are only 0.32 and −0.30 kcal mol^{-1}, respectively). As well, the pattern of variation of $\Delta G_d^{\ddagger}$ with increasing chain unsaturation (Figure 4) appears quite different for the present lipid vesicle system than for the recombinant HDL system. These discrepancies suggest that there are significant differences in the nature of the transition state, if not in the actual mechanism, for desorption of phospholipids from the surfaces of lipid vesicles *vs* recombinant HDL particles. The generality of this distinction remains unclear, as some previous studies of lipid probe desorption from other natural or model lipoprotein systems have shown patterns of variation of $\Delta G_d^{\ddagger}$ with lipid structure that quantitatively more closely resemble that observed in the present study (Massey *et al.*, 1982a,b, 1984).

The present results largely support previous suggestions that spontaneous interbilayer transfer of most naturally occurring phospholipids will be much slower than processes such as intracellular "membrane traffic" as a mechanism for intermembrane lipid transport. Nonetheless, the findings reported here may have some interesting biological implications. To cite one example, as we have discussed previously (Silvius & Zuckermann, 1993), macromolecules anchored to artificial or natural membranes by diacyl phospholipids with C_{16}, C_{18} and/or C_{20} (unsaturated) chains may be able to desorb from the membrane surface with half-times of a few hours or tens of hours, a time scale of potential biological significance [e.g., for transfer of some phosphatidylinositol/glycan-anchored proteins (Rifkin & Landsberger, 1990; Cross, 1990) between different cell surfaces]. In a second example, we note that the cardiolipin found in the inner membrane of mammalian mitochondria, uniquely among mammalian membrane phospholipids, is very highly enriched in polyunsaturated acyl chains, a fact that appears to be of functional importance (Colbeau *et al.*, 1971; Daum, 1985). Our present data indicate that di(polyunsaturated) *double*-chain phospholipids would equilibrate spontaneously between membranes with a half-time of a few hours or less, largely precluding

their efficient localization to any single membrane compartment. The four-chain structure of cardiolipin circumvents this problem (the predicted half-time for spontaneous desorption of tetralinoleoyl cardiolipin, calculated assuming that ΔG_d^* for this species is roughly double that for a simple dilinoleoyl phospholipid, is $\geq 10^{15}$ h!), thus allowing this species to remain stably localized to the mitochondrial inner membrane even if all of its acyl chains are polyunsaturated. The constraints imposed by the possibility of spontaneous interbilayer phospholipid transfer may thus prove to be a significant element in the "design principles" underlying the construction of biological (as well as artificial) membranes that must maintain their compositional integrity and distinctness over long periods of time.

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