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The Relationship between Permeant Size and Permeability in Lipid Bilayer Membranes

T.-X. Xiang, B.D. Anderson
Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah 84112

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Abstract. Permeability coefficients (P_m) across planar egg lecithin/decane bilayers and bulk hydrocarbon/water partition coefficients ($K_{w\to hc}$) have been measured for 24 solutes with molecular volumes, V, varying by a factor of 22 and P_m values varying by a factor of 10^7 to explore the chemical nature of the bilayer barrier and the effects of permeant size on permeability. A proper bulk solvent which correctly mimics the microenvironment of the barrier domain was sought. Changes in $P_m/K_{w\to hc}$ were then ascribed to size-dependent partitioning and/or size-dependent diffusivity. The diffusion coefficient-size dependency was described by $D_{barrier} = D_o/V^n$. When *n*-decane was used as a reference solvent, the correlation between log $P_m/K_{w\to hc}$ and log V was poor ($r = 0.56$) with most of the lipophilic (hydrophilic) permeants lying below (above) the regression line. Correlations improved significantly ($r = 0.87$ and 0.90, respectively) with more polarizable solvents, 1-hexadecene and 1,9-decadiene. Values of the size selectivity parameter n were sensitive to the reference solvent ($n = 0.8 \pm 0.3$, 1.2 ± 0.1 and 1.4 ± 0.2, respectively, for decane, hexadecene, and decadiene). Decadiene was selected as the most suitable reference solvent. The value for n in bilayer transport is higher than that for bulk diffusion in decane ($n = 0.74 \pm 0.10$), confirming the steep dependence of bilayer permeability on molecular size. Statistical mechanical theory recently developed by the authors suggests that a component of this steep size dependence may reside in size-dependent solute partitioning into the ordered chain region of bilayers. This theory, combined with the above diffusion model, yielded the relationship, $P_m/K_{w\to hc} = D_o\exp(-\alpha V)V^n$. A fit of the experimental data to this model gave the best fit ($r = 0.93$) with $\alpha = 0.0053 \pm 0.0021$ and $n = 0.8 \pm 0.3$, suggesting that both diffusion and partitioning mechanisms may play a role in determining the size dependence of lipid bilayer permeabilities.

Key words: Permeability — Transport — Bilayers — Size dependence — Partition coefficients — Diffusion coefficients

Introduction

Unlike diffusion in a continuous fluid medium, which exhibits a relatively small dependence on the volume of the diffusing molecule ($D \alpha V^{-n}$ where $n \approx 2/3$ (Wilke & Chang, 1955; Hayduk & Buckley, 1972; Hildebrand, 1977)), a large body of evidence suggests that permeabilities in biological membranes and lipid bilayers exhibit a very steep dependence on diffusant size (Lieb & Stein, 1986; Walter & Gutknecht, 1986; Anderson & Raykar, 1989). By analogy with diffusivities in polymers, which also exhibit a higher sensitivity to the size of the permeant (Lieb & Stein, 1969), the size selectivity observed in lipid bilayers and biomembranes has traditionally been viewed as having its molecular origin in the effects of lipid chain ordering on diffusion (Stein, 1986; Walter & Gutknecht, 1986). Stein and coworkers were the first to propose that biological membranes behave as polymeric networks with respect to the diffusion of nonelectrolytes (Lieb & Stein, 1969, 1971). More recently, Walter and Gutknecht (1986) have compiled a relatively large database of permeability coefficients for nonelectrolytes and have treated the effects of permeant size on bilayer permeability in the framework of the hypothesis proposed by Stein and coworkers. Although the rather large scatter in their data due to the variation of experimental methods used by different laboratories prevented them from drawing quantitative conclusions regarding the permeant size—permeability relationship, they noted a steeper size dependence for very small molecules than that for medium-sized molecules.

Correspondence to: B.D. Anderson

According to the "solubility-diffusion" model for membrane transport, permeability coefficients are related to the product of the partition coefficient of the permeant between the membrane barrier domain and water, K_m, and the normal component of the diffusion coefficient within the barrier region, D_m. While lateral diffusivities within bilayers (Almeida, Vaz & Thompson, 1992; Vaz, Clegg & Hallmann, 1985) and local microviscosities (Chen et al., 1977; Brown, Ribeiro & Williams, 1983; Pfeiffer et al., 1988) are routinely measured, diffusion coefficients in the normal direction are not available except in liquid crystals (Moscicki, Shin & Freed, 1993). Walter and Gutknecht (1986) argued that size effects on permeant partitioning into the bilayer interior are unimportant, however, on the basis of the similarity in the solubilities of small *n*-alkanes (up to *n*-butane) in lipid bilayers (Miller, Hammond & Porter, 1977; Simon, Stone, Busto-Latorre, 1977) and it is now common practice to assume that size-dependent permeabilities are explained solely by changes in the diffusion coefficient (Finkelstein, 1976; Walter, 1981; Walter & Gutknecht, 1986).

It is well known, however, that the solubilities of higher alkanes in lipid bilayers exhibit substantial decreases with increasing chain length (White, 1977, 1978). Moreover, statistical mechanical theory recently developed in the authors' laboratories suggests a strong molecular size dependence for permeant partitioning into the rate-limiting barrier domain, which is assumed to be in the highly ordered chain region (Xiang & Anderson, 1994). Therefore, the size selectivity for transport across lipid bilayers may be a combined result of size-dependent diffusion and partitioning.

Although membrane/water partition coefficients can be measured directly, they may have limited value in predicting relative permeability coefficients if the domain probed in partitioning studies is not the rate-limiting domain for transport. Lipid bilayers are heterogeneous systems which can be divided roughly into three distinct regions: an ordered, highly polar interfacial (head group) region, a highly ordered hydrocarbon chain region, and a region of relatively disordered hydrocarbon chains near the center of the bilayer. Each region has its own chemical and diffusional properties (Diamond, Szabo & Katz, 1974; Xiang, 1993). To predict the effects of permeant size on permeability across a lipid bilayer, it is therefore essential to choose a reference solvent system which mimics as closely as possible the chemical microenvironment of the barrier domain. Historically, various solvents have been utilized as reference solvents but the prevailing view is that alkane solvents (i.e., hexadecane) most closely resemble the partitioning behavior of the permeability barrier in lipid bilayers (Finkelstein, 1976; Lieb & Stein, 1986; Walter & Gutknecht, 1986). However, as noted in a separate study recently conducted in our laboratories of functional group contributions to lipid bilayer permeability (Xiang, Chen & Anderson, 1992; T.-X. Xiang and B.D. Anderson, *submitted*) hexadecane does not mimic the barrier microenvironment for the transport of nonelectrolytes over a wide range of lipophilicity as well as more polarizable hydrocarbon solvents (e.g., hexadecene or 1,9-decadiene).

The primary objective of this study was to examine the size dependence of lipid bilayer permeability in a more systematic manner. We approached this problem by measuring solute fluxes across egg lecithin/decane planar lipid bilayers and bulk hydrocarbon solvent/water partition coefficients for 24 permeants with molecular sizes differing by a factor of 22 and permeability coefficients differing by a factor of 10^7. The experimental results were analyzed by the conventional approach which assumes only size-dependent diffusion coefficients and by a model which combined the effects of solute size on diffusivity and on partitioning in the bilayer interior using our recently developed statistical mechanical theory (Xiang & Anderson, 1994).

Materials and Methods

Materials

Egg lecithin in chloroform obtained from Avanti Polar Lipids (Pelham, AL) was dried under nitrogen gas and dispersed in decane which had been passed through an alumina column before use. Four radiolabeled compounds, ^{3}H-water, ^{14}C-formic acid, ^{3}H-acetic acid and ^{3}H-acetamide were used. ^{3}H-water, ^{14}C-formic acid and ^{3}H-acetic acid were purchased from ICN Biomedicals (Costa Mesa, CA). ^{3}H-acetamide was synthesized in our laboratory by reaction of 10 mmol ^{3}H-acetic anhydride (New England Nuclear, Boston, MA) with 60 mmol NH_4OH (30% in H_2O) and the crude product was purified by HPLC. The unlabeled compounds, α-substituted *p*-toluic acids and 21-[(7-amino-1,7-dioxoheptyl)oxy]-11,17-dihydroxy-pregn-4-ene-3,20,dione (hc-21-pimelamide), were synthesized previously (Raykar, Fung & Anderson, 1988; T.-X. Xiang and B.D. Anderson, *submitted*). ddA (2′,3′-dideoxyadenosine) and ddI (2′,3′-dideoxyinosine) with reported purities of 99% were supplied by the National Cancer Institute. All other compounds were obtained commercially and used without further purification.

Lipid Bilayer Permeability Coefficients

Detailed descriptions of the transport experiments have been reported previously (Xiang et al., 1992). Briefly, lipid bilayers were formed by applying a phospholipid solution (2% w/v, egg lecithin in decane) across a 1 mm diameter hole in a Teflon partition separating two water-jacketed chambers each containing ≈4.5 ml aqueous buffer. Both chambers were stirred continuously with magnetic fleas and maintained at 25.0 ± 0.05°C during the transport experiments. The ionic strength was held at I = 0.1 with NaCl and the pH in both cham-

bers was monitored with a microcombination pH probe (MI-421, Microelectrodes, Londonderry, NH).

The thinning of the lipid membrane to a bilayer thickness of ≈50 Å was monitored by capacitance measurements (Xiang et al., 1992). After a constant capacitance was attained, a small amount (≈10 − 40 μl) of solution containing permeant at a known concentration was injected into the donor solution and a corresponding volume of buffer was added to the receiver solution. Samples were withdrawn from both chambers at various time intervals. Samples containing radioactive permeants were mixed with Aquasol scintillation cocktail and counted in a liquid scintillation counter (Beckman LS 1801, Beckman Institute, Fullerton, CA). Unlabeled permeants were analyzed by HPLC.

Apparent permeability coefficients were calculated from the slopes of plots of receiver concentration *vs.* time interval Δt and bilayer area, volume of the aqueous solution in both chambers and solute concentration in the donor solution (Xiang et al., 1992). Point-by-point areas were monitored continuously during the experiments either microscopically or by the capacitance method described previously. Apparent permeability coefficients were calculated using the average area over each time interval. The absolute area of the Teflon hole in which bilayers were formed was determined with vernier microcalipers. Microscopic determinations of the areas of the "black" (bilayer) portion of lipid films during the transport experiments were made using a StereoZoom microscope (Bausch and Lomb, Rochester, NY) aligned normal to the membrane. Photographs were taken with a 3-1/4 × 4-1/4″ Polaroid Land camera (Bausch and Lomb, Rochester, NY). The membrane was transilluminated with an illuminator (M 650, Reichert Scientific Instruments, Buffalo, NY). After photographing the membrane, the area of the bilayer was determined by the weight-area method.

Bulk Organic Solvent/Water Partition Coefficients

Partition coefficients were measured using the shake flask method. The organic solvents were washed three times with roughly equal volumes of water before the partition experiments. A 1 ml aqueous solution of the desired solute was filtered and placed in a 5–10 ml centrifuge tube. A 1–5 ml portion of organic solvent was then added. After vortex mixing for 5 min and centrifugation, the sample was allowed to stand in a water bath (25.0 ± 0.05°C) for 5 hr. Aliquots of the sample were carefully withdrawn from both phases and appropriately diluted or enriched for subsequent concentration analyses either by scintillation counting or HPLC. For ionizable species, the pH in the aqueous phase was maintained well below the solute's pK_a value so that more than 99% of the solute was in its nonionized form. Hydrocarbon/water partition coefficients were calculated using molar concentrations.

pK_a Determinations

The pK_a values for α- and β-naphthoic acid were determined by the solubility-pH method. Five samples, each having 2 ml of 0.05 M acetic acid buffer at a given pH between 3.0 and 5.5, were placed in a bath at 25 ± 0.05°C. Excess solid was added and the mixtures were shaken. When equilibrium was reached, aliquots were withdrawn from the solution, passed immediately through 0.45 μm filters equilibrated at the same temperature, and their pH values were measured. The filtrates were then diluted appropriately and assayed by HPLC. The pK_a values were determined from least-squares fits of the solubility (S)—pH data with the equation

$$S = [HA]_m(1 + K_a/[H^+]) \quad (1)$$

where $[HA]_m$ is the solubility of an unionized free acid.

HPLC Analyses

An HPLC system consisting of a syringe-loaded sample injector (Rheodyne Model 7125, Rainin Instruments, Woburn, MA) with 100 μl loop, a solvent pump (Model 110A, Beckman Instruments, Fullerton, CA), a dual-wavelength absorbance detector (Model 441, Water Associates, Milford, MA) operated at 254 nm, an integrator (Model 3392A, Hewlett-Packard, Avondale, PA), and a reversed-phase column packed with 5 μm Spheri-5 RP-18 (Brownlee OD-MP, 4.6 mm ID × 10 cm, Rainin) was used at ambient temperature for the analyses of the unlabeled solutes in samples taken from the transport and diffusion experiments. The mobile phase contained acetonitrile (5–40%) in deionized water and was buffered to a pH of 3.0 for the weak acids or a pH of 7.0 for the nonionizable solutes using 0.01 M phosphate buffer.

Results

Bulk Organic Solvent/Water Partition Coefficients

Partition coefficients between *n*-decane, 1-hexadecene or 1,9-decadiene and water at 25°C are presented in Table 1. The apparent partition coefficients ($K_{app} = C_{hc}/C_w$, where C_{hc} and C_w are, respectively, the molar concentrations of solute in the hydrocarbon solvent and in water) of the carboxylic acids examined in this study were found to vary with solute concentration as shown in Fig. 1 for the partitioning of benzoic acid, α-naphthoic acid, and 9-anthroic acid between decane and water. This behavior is attributed to self-association of carboxylic acids in the nonpolar hydrocarbon solvent (Pimental & McClelland, 1960). This concentration dependence can be described by the following thermodynamic relationship between K_{app} and C_w,

$$K_{app} = C_{hc}/C_w = K_{w\to hc}(1 + nK_n K_{w\to hc}^{n-1} C_w^{n-1}) \quad (2)$$

assuming that a monomer-single polymer equilibrium (K_n) model is applicable and that self-association in the aqueous phase is negligible. Referring to Fig. 1, the data for benzoic acid and α-naphthoic acid are well described by a monomer-dimer equilibrium model ($n = 2$) while the apparent partition coefficient for 9-anthroic acid appears to be independent of C_w below 10^{-4} M, reflecting the probable effects of steric hindrance. Dimerization constants of (2.1 ± 0.4) × 10^4 M^{-1} and (2.5 ± 0.4) × 10^4 M^{-1} were obtained from the fits for benzoic acid and α-naphthoic acid. The dimerization constant

Table 1. *n*-Decane, 1-hexadecene and 1,9-decadiene/water partition coefficients (molar concentrations) for various solutes at 25°C

Permeant	Partition coefficient		
	Decane/water	Hexadecene/water	Decadiene/water
1 Water	$(2.0 \pm 0.2) \times 10^{-5}$	$(5.9 \pm 0.2) \times 10^{-5}$	$(1.2 \pm 0.1) \times 10^{-4}$
2 Formic acid	$(1.8 \pm 0.1) \times 10^{-4}$	$(3.0 \pm 0.6) \times 10^{-4}$	$(6.2 \pm 0.4) \times 10^{-4}$
3 Acetic acid	$(3.1 \pm 0.5) \times 10^{-4}$	$(7.8 \pm 0.2) \times 10^{-4}$	$(1.3 \pm 0.2) \times 10^{-3}$
4 Acetamide	9.3×10^{-6}	$(3.8 \pm 0.5) \times 10^{-5}$	$(1.3 \pm 0.2) \times 10^{-4}$
5 Butyric acid	$(9.5 \pm 0.4) \times 10^{-3}$	$(1.8 \pm 0.1) \times 10^{-2}$	$(3.9 \pm 0.1) \times 10^{-2}$
6 Adenine	$(3.7 \pm 0.1) \times 10^{-7}$	$(3.1 \pm 0.5) \times 10^{-6}$	$(5.8 \pm 1.7) \times 10^{-6}$
7 Benzoic acid	$(4.9 \pm 0.3) \times 10^{-2}$	$(1.5 \pm 0.1) \times 10^{-1}$	$(3.1 \pm 0.1) \times 10^{-1}$
8 *p*-Toluic acid	$(2.0 \pm 0.1) \times 10^{-1}$	$(5.2 \pm 0.1) \times 10^{-1}$	$(9.0 \pm 0.1) \times 10^{-1}$
9 α-Hydroxy-*p*-toluic acid	$(5.0 \pm 0.2) \times 10^{-5}$	$(2.5 \pm 0.1) \times 10^{-4}$	$(7.3 \pm 0.7) \times 10^{-4}$
10 α-Chloro-*p*-toluic acid	$(2.8 \pm 0.3) \times 10^{-1}$	$(4.2 \pm 0.1) \times 10^{-1}$	$(5.3 \pm 2.4) \times 10^{-1}$
11 α-Cyano-*p*-toluic acid	$(3.7 \pm 0.1) \times 10^{-3}$	$(9.6 \pm 1.9) \times 10^{-3}$	$(1.7 \pm 0.1) \times 10^{-2}$
12 α-Methoxy-*p*-toluic acid	$(4.3 \pm 1.2) \times 10^{-2}$	$(6.4 \pm 0.5) \times 10^{-2}$	$(1.1 \pm 0.1) \times 10^{-1}$
13 α-Naphthoic acid	$(6.9 \pm 0.4) \times 10^{-1}$	2.0 ± 0.2	2.2 ± 0.2
14 β-Naphthoic acid	2.0 ± 0.1	4.1 ± 0.1	5.7 ± 0.2
15 α-Carboxy-*p*-toluic acid	$(1.4 \pm 0.2) \times 10^{-5}$	$(4.0 \pm 0.2) \times 10^{-5}$	$(1.2 \pm 0.2) \times 10^{-4}$
16 α-Carbamido-*p*-toluic acid	$(1.8 \pm 0.3) \times 10^{-6}$	$(7.0 \pm 0.6) \times 10^{-6}$	$(4.0 \pm 0.7) \times 10^{-5}$
17 9-Anthroic acid	1.2 ± 0.1	3.0 ± 0.1	6.4 ± 0.5
18 2′,3′-Dideoxyadenosine	$(3.6 \pm 0.2) \times 10^{-6}$	$(5.0 \pm 0.1) \times 10^{-5}$	$(6.4 \pm 1.1) \times 10^{-5}$
19 2′-Deoxyadenosine	$(8.0 \pm 0.4) \times 10^{-8}$	6.0×10^{-7}	$(2.4 \pm 0.9) \times 10^{-6}$
20 Prednisolone	$(1.5 \pm 0.2) \times 10^{-5}$	$(1.6 \pm 0.1) \times 10^{-4}$	$(7.9 \pm 1.0) \times 10^{-4}$
21 Hydrocortisone	$(6.6 \pm 0.1) \times 10^{-5}$	$(5.1 \pm 0.8) \times 10^{-4}$	$(1.75 \pm 0.05) \times 10^{-3}$
22 hc-21-pimelamide	—	$(2.44 \pm 0.01) \times 10^{-4}$	$(1.3 \pm 0.1) \times 10^{-3}$

for benzoic acid in hexane has been previously reported to be 2.1×10^4 M (Takeda, Yamashita & Akiyama, 1987). The intrinsic bulk solvent/water partition coefficients, $K_{w \to hc}$, determined in this manner, are the values presented in Table 1.

Lipid Bilayer Permeability Coefficients

To properly correct for the effects of permeant lipophilicity on the size dependence of permeability, permeant molecules with different lipophilicity were utilized. The more lipophilic permeants included compounds having a single carboxylate group attached to an aliphatic chain or an aromatic ring. Because many of these permeants have high membrane permeabilities, the method of pH adjustment (Gutknecht & Tosteson, 1973; Walter, Hastings & Gutknecht, 1982; Walter & Gutknecht, 1984; Xiang et al., 1992) was used to identify for each permeant a pH "window" in which transport is membrane, rather than unstirred water layer controlled. In the absence of boundary layer pH gradients and assuming that only the neutral species diffuse across the bilayer, one can relate the apparent permeability (P_{app}) to the fraction of unionized species (f_{HA}) by

$$\frac{1}{P_{app}} = \frac{1}{P_m f_{HA}} + \frac{1}{P_{aq}} \qquad (3)$$

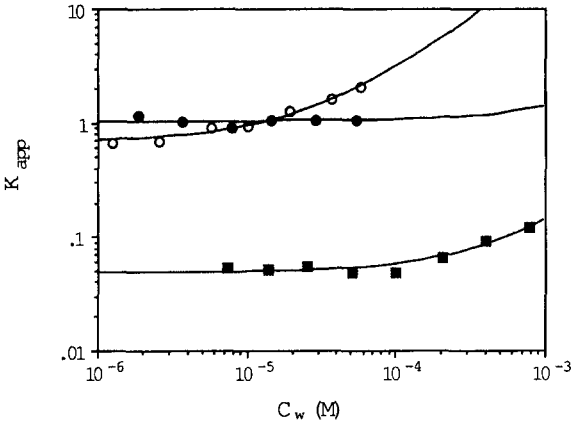

Fig. 1. Representative plots of apparent partition coefficients (K_{app}) in decane/water for benzoic acid (■), α-naphthoic acid (○) and 9-anthroic acid (●) as a function of their aqueous concentration, C_w, at 25°C.

where P_{aq} is the permeability coefficient across the unstirred layer. Values of f_{HA} were determined at each pH from pK_a values which were available in the literature or measured by these investigators (T.-X. Xiang and B.D. Anderson, *submitted;* or this study). P_{aq} was assumed to be independent of solution pH as both charged and neutral species have nearly the same diffusion coefficients in aqueous solution (Albery, Greenwood & Kibble, 1967; Xiang & Anderson, 1993). Regression

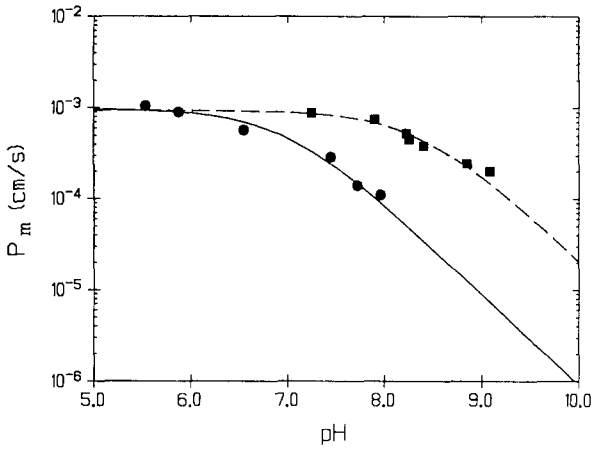

Fig. 2. Permeability—pH profiles for α-naphthoic acid (●) and β-naphthoic acid (■) at 25°C. Curves are nonlinear least-squares fits using Eq. (3).

analyses of the P_{app}—pH profiles according to Eq. (3), illustrated in Fig. 2 for α- and β-naphthoic acids, yielded estimates of both P_{aq} and P_m. For α- and β-naphthoic acid, values of f_{HA} were determined from measured pK_a values, corrected for ionic strength, of 3.85 ± 0.06 and 4.08 ± 0.02, respectively (literature values were 3.70 and 4.17, respectively (Hodgman et al., 1963)). As expected, the apparent permeability coefficients were pH independent at low pH and virtually independent of permeant structure, reflecting unstirred layer controlled transport (P_{aq}). P_m values were obtained from the pH region in which apparent permeability coefficients decreased with increasing pH (i.e., as the fraction of neutral species decreased), indicating that the transport was bilayer membrane controlled.

The permeability coefficients obtained in this work, listed in Table 2, vary by a factor of 10^7. Estimates of upper limits for the permeability coefficients of ddI and ddG were determined from the known sensitivity of the assay and our failure to detect transported ddI and ddG. The permeability coefficient for water, $P_m = (1.9 \pm 0.9) \times 10^{-3}$ cm/sec, was calculated from the observed apparent permeability, $P_{app} = (9.8 \pm 0.7) \times 10^{-4}$ cm/sec, according to, $1/P_m = 1/P_{app} - d_{aq}/D_w$. D_w is the self-diffusion coefficient of water, 2.45×10^{-5} cm²/sec (Robinson & Stokes, 1959) and d_{aq} is the sum of the unstirred water layer thicknesses on the two sides of the membrane. The value of d_{aq} used in obtaining P_m for water was the average d_{aq} (120 ± 40 μ) determined experimentally from the P_{app}—pH profiles of several ionizable permeants.

Selection of a Model Solvent

Constraints imposed by interfacial forces in bilayers result in partial chain ordering and a variation in molecular organization with depth from the surface (Seelig & Seelig, 1974; Dill & Flory, 1981). Solutes are excluded from regions of high local order, concentrating preferentially in the more disordered midbilayer region (White, King & Cain, 1981; Marqusee & Dill, 1986). In a similar fashion, free volume is distributed nonuniformly with depth (Xiang, 1993) possibly leading to gradients in diffusion coefficients (Barenholz et al., 1991). Taking membrane heterogeneity into account, the permeability coefficient (P_m) can be expressed in terms of $K_{w\to z}$ and D_z, both functions of depth (z), by the following kinetic integral (Diamond & Katz, 1974)

$$\frac{1}{P_m} = \int_0^d \frac{dz}{K_{w\to z} D_z} \tag{4}$$

where d is the thickness of the membrane. If, on the other hand, permeation is rate-limited by a distinct region within the bilayer (e.g., the ordered chain region as depicted in Fig. 3) with an effective thickness of δ, the above equation reduces to

$$\frac{P_m \delta}{K_{w\to hc}} = D_{barrier} \xi \tag{5}$$

where ξ represents the size selectivity for solute parti-

Table 2. Permeability coefficients (P_m) for various nonelectrolyte solutes across egg lecithin bilayer membranes at 25°C

	Permeant	V (Å³)[a]	P_m (cm/sec)
1	Water	20.6	$(1.9 \pm 0.9) \times 10^{-3}$
2	Formic acid	38.5	$(2.9 \pm 0.1) \times 10^{-3}$
3	Acetic acid	55.5	$(5.0 \pm 0.2) \times 10^{-3}$
4	Acetamide	59.7	$(2.9 \pm 0.3) \times 10^{-4}$
5	Butyric acid	89.5	$(1.0 \pm 0.2) \times 10^{-1}$
6	Adenine	107	$(1.38 \pm 0.02) \times 10^{-5}$
7	Benzoic acid	108	$(5.7 \pm 0.5) \times 10^{-1}$
8	*p*-Toluic acid	125	1.1 ± 0.2
9	α-Hydroxy-*p*-toluic acid	133	$(1.6 \pm 0.4) \times 10^{-3}$
10	α-Chloro-*p*-toluic acid	139	$(6.4 \pm 0.1) \times 10^{-1}$
11	α-Cyano-*p*-toluic acid	144	$(2.7 \pm 0.5) \times 10^{-2}$
12	α-Methoxy-*p*-toluic acid	148	$(3.5 \pm 0.1) \times 10^{-1}$
13	β-Naphthoic acid	149	$(1.7 \pm 0.2) \times 10^{1}$
14	α-Naphthoic acid	149	2.3 ± 0.6
15	α-Carboxy-*p*-toluic acid	152	$(1.8 \pm 0.3) \times 10^{-4}$
16	α-Carbamido-*p*-toluic acid	157	$(4.1 \pm 0.4) \times 10^{-5}$
17	9-Anthroic acid	190	3.2 ± 0.8
18	2′,3′-Dideoxyadenosine	195	$(6.3 \pm 0.1) \times 10^{-5}$
	2′,3′-Dideoxyinosine		$<10^{-6}$
	2′,3′-Dideoxyguanine		$<10^{-6}$
19	2′-Deoxyadenosine	203	$(9.4 \pm 0.7) \times 10^{-7}$
20	Prednisolone	309	$(1.5 \pm 0.6) \times 10^{-4}$
21	Hydrocortisone	316	$(5.6 \pm 0.3) \times 10^{-4}$
22	Hydrocortisone-21-pimelamide	452	$(1.8 \pm 0.5) \times 10^{-4}$

[a] Molecular volume calculated by the atomic increment method (Edward, 1970).

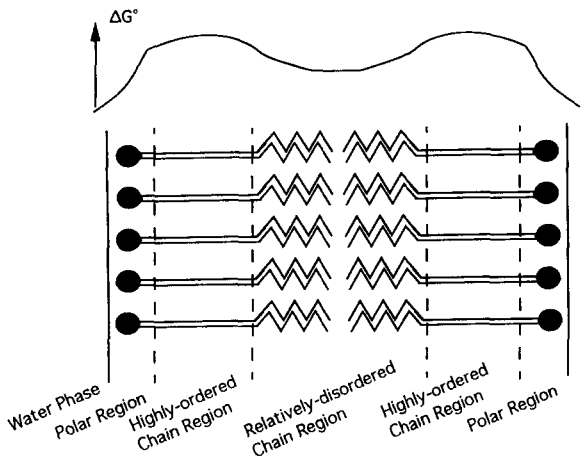

Fig. 3. A schematic illustration of the three distinct regions within a lipid bilayer. The free energy plot serves as a qualitative indicator of the barrier properties in the lipid bilayer.

tioning into the bilayer barrier domain relative to that between the hydrocarbon chosen as a reference solvent and water. The above expression is valid only if the reference solvent chosen appropriately describes the chemical environment of the barrier domain. In the following analysis, the effective thickness of the barrier domain, δ, was assumed to be the same for all solutes studied, with a value of 18 Å.

Assuming that the rate-limiting region in the bilayer behaves like a continuous liquid medium, an equation having the form of the Stokes-Einstein relation is often assumed for the diffusion coefficient in the barrier region, D_{barrier}

$$D_{\text{barrier}} = \frac{D_o}{V^n} \tag{6}$$

where D_o is a constant depending on the microviscosity in the barrier domain and V is the molecular volume (V) of the diffusing permeant. Molecular volumes were calculated by the atomic increment method (Edward, 1970)

$$V = \sum_{i=1}^{N} l_i V_i \tag{7}$$

where there are N atomic species in the chemical formula, and l_i atoms of type i having atomic volumes of V_i. Although n was originally assumed to be one-third for spherical molecules diffusing in continuous liquid media, Hildebrand (1977) suggested that the diffusion coefficient depends mainly on the cross-sectional area of the permeant, or $n = 2/3$. Wilke and Chang (1955) also concluded from experimental data in bulk liquids that n is close to 0.6.

In the absence of size-dependent partitioning (ξ = 1), Eqs. (5) and (6) can be combined to yield the following relationship

$$\log\frac{P_m\delta}{K_{\text{w}\to\text{hc}}} = \log D_o - n\log V \tag{8}$$

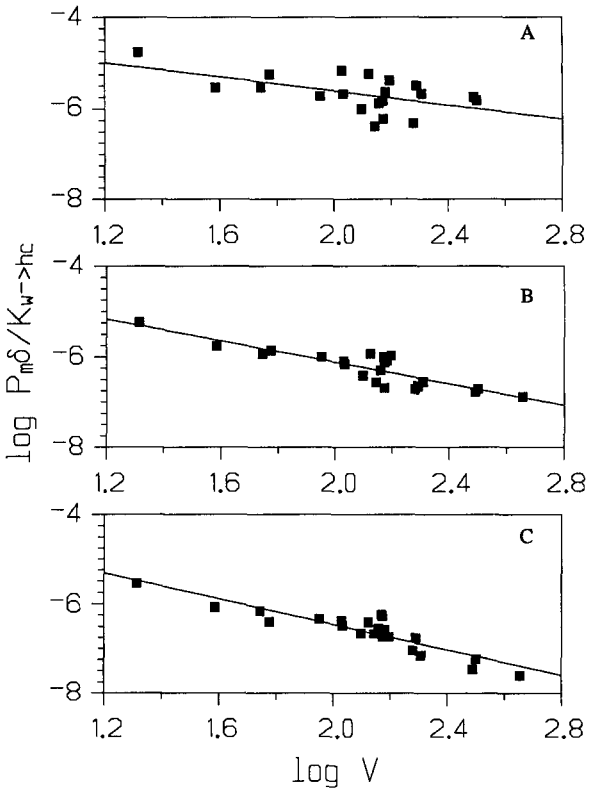

Fig. 4. Plots of log $P_m\delta/K_{\text{w}\to\text{hc}}$ *vs.* log of permeant volume (Å^3/molecule), using *n*-decane (*A*), 1-hexadecene (*B*), or 1,9-decadiene (*C*) as reference solvents. The unbroken lines are linear least-squares fits according to Eq. (8).

In Fig. 4*A-C*, the logarithms of $P_m\delta/K_{\text{w}\to\text{hc}}$ are plotted against log V, with $K_{\text{w}\to\text{hc}}$ representing the partition coefficients between *n*-decane (Fig. 4*A*), 1-hexadecene (Fig. 4*B*), or 1,9-decadiene (Fig. 4*C*) and water, respectively. The parameter and statistical values generated from regression analyses of these data according to Eq. (8) are listed in Table 3.

Using decane as a reference solvent (Fig. 4*A*), the data are relatively scattered ($r = 0.56$) with the more hydrophilic (lipophilic) permeants lying above (below) the regression line. This correlation coefficient is similar to that obtained by Walter and Gutknecht (1986) using *n*-hexadecane as a reference solvent to analyze the relationship between permeant size and permeability for 23 permeants ($r = 0.67$; $n = 1.2$). The correlations improved significantly ($P < 0.05$, F-test) when more polar/polarizable hydrocarbon solvents were used as reference solvents, as indicated by the results in Fig. 4*B*

Table 3. Parameter and statistical values derived from Eqs. (8) or (11) to account for the size selectivity for transport across lecithin bilayers

Solvent	n	Intercept	α	r^{a}
n-Decane	0.8 ± 0.3	−4.1 ± 0.6		0.56
1-Hexadecene	1.2 ± 0.1	−3.7 ± 0.3		0.87[b]
1,9-Decadiene	1.4 ± 0.2	−3.6 ± 0.3		0.90[b]
1,9-Decadiene	0.8 ± 0.3	−4.7 ± 0.5	0.0053 ± 0.002	0.93[b]
1,9-Decadiene		−5.9 ± 0.1	0.010 ± 0.001	0.90[b]

[a] Square root of the coefficient of determination.
[b] Significantly improved fit ($P < 0.05$, F-test for equality of variances) over the model using decane partition coefficients.

and C, where 1-hexadecene and 1,9-decadiene were used, respectively.

The relatively poor fit when decane is used as a reference solvent and that significantly improved fits are obtained with 1-hexadecene and 1,9-decadiene suggest that the lipid bilayer barrier domain is more polar/polarizable than an alkane solvent. However, it is well known that partitioning between water and different solvents or biomembranes can be correlated by linear free energy relationships as long as the differences in the partitioning behavior of the solvents and membranes are small (Collander, 1951; Diamond & Katz, 1974). This may hold in our present analysis as no strong interactions such as hydrogen bonding exist between the permeants and the three model solvents used. Applying this principle, one can correlate permeability coefficients with both partition coefficients and solute molecular volumes by

$$\log P_m = \log D_o/\delta + s\log K_{w\to hc} - n\log V \qquad (9)$$

The selectivity coefficient, s, provides information on the degree to which the lipid bilayer resembles the solvent properties of the reference solvent used. The most appropriate reference solvent yields an s value equal to one. Multiple regression analyses of the permeability data according to Eq. (9) resulted in no significant improvement in fit for the 1-hexadecene and 1,9-decadiene data, but the decane data were fit significantly better ($P < 0.05$) when compared to the fit using Eq. (8). There were no longer any statistically significant differences in the regressions using the various model solvents when s was included as an adjustable parameter. Values of the selectivity coefficient, s, were 0.88 ± 0.02, 0.97 ± 0.02, and 1.03 ± 0.02 for decane, 1-hexadecene, and 1,9-decadiene, respectively, confirming the previous finding that the more polarizable hydrocarbon solvents more closely resemble the partitioning behavior of lipid bilayer membranes. The values obtained for n, which differ significantly depending on the reference solvent, were not appreciably altered by the inclusion of s as an adjustable parameter (n = 0.7 ± 0.2, 1.2 ± 0.1, and 1.4 ± 0.2, for decane, 1-hexadecene, and 1,9-decadiene, respectively).

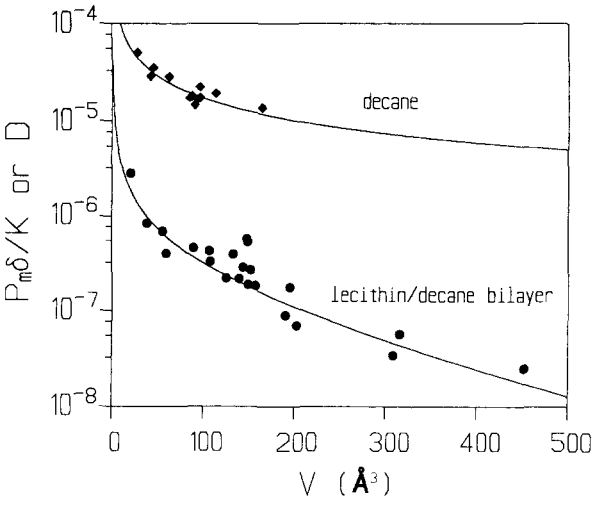

Fig. 5. Plot of log D for diffusion of various solutes in decane (Walter & Ioakimidis, 1976; Evans, Tominaga & Chan, 1979) *vs.* molecular volume, V, (Å^3/molecule) (upper curve) in comparison to the plot of log $P_m\delta/K_{w\to hc}$ *vs.* V using 1,9-decadiene as a reference solvent. The unbroken curves are best fits of the data using Eq. (6) (upper curve) or Eq. (11) (lower curve).

Permeability—Permeant Size Relationships

We now focus attention on the proper form for the relationship between diffusion coefficients in lipid bilayer membranes and permeant size. Figure 5 shows the logarithms of the diffusion coefficients of several nonelectrolytes in bulk decane at 298 K as a function of molecular volume along with the lecithin/decane bilayer permeability data corrected for the assumed barrier domain thickness and normalized by decadiene/water partition coefficients. A fit of the decane diffusion data to an equation having the same form as Eq. (6) gave n = 0.74 ± 0.10, close to the two-thirds dependence suggested by Hildebrand (1977) for diffusion in bulk solvents, but about half that obtained for permeation in the bilayer (n = 1.4) when the appropriate (i.e., 1,9-decadiene) reference solvent is used.

The steep size dependence for transport across biological membranes is generally attributed to the effects of chain ordering on the diffusion coefficient (Lieb & Stein, 1986), but this ignores the possibility that partitioning into bilayers is also likely to exhibit a dependence on solute size due to the unfavorable configurational entropy and high lateral pressures in the highly ordered chain region of bilayers (Marqusee & Dill, 1986; Xiang & Anderson, 1994). We have recently developed a mean-field statistical mechanical theory for

molecular partitioning into interphases (Xiang & Anderson, 1994). The excluded volume interaction is modeled in terms of a reversible work which is required to create a cavity for the solute having size V in an interphase against a pressure tensor exerted by the surrounding interphase molecules. Model calculations show that increased lateral pressures $p_\perp$ (bar) result in solute exclusion from the interphase. Provided that the reference solvent mimics closely the chemical microenvironment of the bilayer barrier domain, the size selectivity ξ for partitioning of globular permeants into the rate-limiting domain is predicted to decrease exponentially with permeant volume

$$\xi = e^{-2(p_\perp - 1)V/3k_BT} \tag{10}$$

where k_B is the Boltzmann constant and T the absolute temperature. Equation (10) is also an excellent approximation for elongated solutes due to the relatively weak dependence of solute partitioning on molecular shape (Xiang & Anderson, 1994).

Including the effects of molecular size on solute partitioning into bilayers in Eq. (8), we obtain the overall predicted effect of permeant size on membrane permeability

$$\log P_m \delta / K_{w\to hc} = \log D_o - n\log V - \alpha V/2.303 \tag{11}$$

where $\alpha = 2/3\ (p_\perp - 1)/k_BT$. Since decadiene was previously selected as a suitable model solvent for the chemical properties of egg phospholipid bilayers, Eq. (11) was applied to the permeabilities divided by decadiene/water partition coefficients, with $\log D_o$, n and α as adjustable parameters. The lower curve in Fig. 5 is the best fit of the data assuming this model. By setting either n or α equal to zero in Eq. (11) it was possible to test models which ascribe the entire molecular size dependence in permeability coefficients to either size-dependent partitioning ($n = 0$) or size-dependent diffusion coefficients ($\alpha = 0$). The regression results are presented in Table 3. Although the coefficient of determination was highest when both size-dependent partitioning and diffusion were allowed, application of the F-test for equality of variances indicated no significant differences in the fits of the $P_m\delta/K_{w\to decadiene}$ data regardless of the form of the size dependence relationship used.

Discussion

Reliability of the Permeability Measurements

As noted by previous investigators (Walter et al., 1982) and evident from an inspection of Eq. (3), the membrane permeabilities of relatively small or lipophilic permeants may be so high that diffusion through unstirred water layers adjacent to the membrane is the rate-limiting step for diffusion (i.e., $P_{aq} \ll P_m$) and the true P_m value cannot be obtained reliably from simple flux measurements. For this reason, we have, where possible (i.e., ionizable permeants) used the method of pH adjustment, described in previous publications (Xiang et al., 1992; Xiang & Anderson, 1993) to identify for each permeant a pH "window" in which transport is clearly membrane controlled, as illustrated in Fig. 2 for α- and β-naphthoic acids. In this manner, P_{aq} values could be determined for each of the ionizable permeants and substituted into Eq. (3) or included as fitted parameters in applying Eq. (3) to the permeability *vs.* pH profiles to generate the P_m values listed in Table 1. P_{aq} values should be independent of lipophilicity and only weakly dependent on molecular volume ($P_{aq}\alpha V^{-1/3}$) through the Stokes-Einstein relationship. Consistent with this expectation, estimates of the unstirred layer thickness, d_{aq}, varied over a narrow range from experiment to experiment (120 ± 40 μ). The variation in d_{aq} from experiment to experiment was due perhaps to differences in hole size in the Teflon sheets used, or to changes in positioning of the hole where the lipid bilayers were formed in relation to the position of the magnetic fleas after cell disassembly and reassembly. With the exception of P_m for water, the unstirred water layer corrections applied to nonionizable permeant P_{app} data were small and not significantly affected by the experiment-to-experiment variation in d_{aq}. The unstirred water layer correction for the diffusion of ^3H_2O is rather large, however, and the uncertainty reported for water's P_m is mainly from the uncertainty in the unstirred layer thickness estimation.

The permeability coefficient for water obtained in this study ($1.9 \pm 0.9 \times 10^{-3}$ cm/sec) agrees reasonably well with values reported by Finkelstein (1976), 2.2×10^{-3} cm/sec, and Walter (1981), 3.4×10^{-3} cm/sec. The permeability coefficients for acetic acid and butyric acid from this study are in close agreement with those observed by Walter and Gutknecht (1984), 6.9×10^{-3} cm/sec and 9.5×10^{-2} cm/sec, respectively, whereas our permeability coefficient for formic acid, $(2.9 \pm 0.1) \times 10^{-3}$ cm/sec, is 2.5 times smaller than the value given by Walter and Gutknecht (1984) of 7.3×10^{-3} cm/sec. Formic acid was not included in their regression analysis of the permeability—size profile because of its anomalously high permeability coefficient.

Some of the values in Table 1, notably those for *p*-toluic acid and its α-substituted analogues were reported elsewhere (T.-X. Xiang and B.D. Anderson, *submitted*). They were used to generate apparent functional group contributions to the free energy of transfer of permeant from water to the bilayer barrier domain. For example, a value of 3.9 kcal/mol was estimated for the

−OH group contribution, indicating that addition of well-isolated primary −OH to a permeant molecule decreases its permeability across egg lecithin bilayers by a factor of ≈600. In contrast to this prediction, a comparison of the permeability coefficients between 2′-deoxyadenosine and 2′,3′-dideoxyadenosine indicates that the addition of an −OH group at position 3′-C decreases permeability by a factor of only 60. This relatively small −OH group effect on permeability suggests the existence of a strong intramolecular interaction of the 3′-OH group with neighboring substituent(s), probably with the 5′-OH group.

On the Selection of a Model Bulk Solvent to Describe the Chemical Nature of the Barrier Domain in Lipid Bilayer Membranes

As originally pointed out by Diamond and Katz (1974), the resistance to permeation across lipid bilayer membranes ($1/P_m$) may be expressed as a sum of interfacial resistances and the resistance offered by the hydrocarbon chain region of the bilayer. The contributions of the bilayer interfacial regions to the overall transport barrier are generally assumed to be negligible, based on the findings of several groups, including our own, that the chemical selectivity of the permeability barrier resembles that of hydrocarbon solvents more closely than more polar, hydrogen bonding solvents which would be closer in properties to the highly polar interfacial region (Finkelstein, 1976; Lieb & Stein, 1986; Walter & Gutknecht, 1986; T.-X. Xiang and B.D. Anderson, *submitted*). Thus, the resistance to permeability in lipid bilayer membranes can be expressed by the integral of the inverse of the products of local partition coefficients ($K_{w\to z}$) and diffusion coefficients (D_z) within the hydrocarbon chain region of the bilayer, both of which are dependent on depth (z), as described in Eq. (4). We have assumed in the present treatment that a distinct region within the bilayer (i.e., the highly ordered chain region) with an effective thickness δ constitutes the rate-limiting region. Both experimental and theoretical evidence justify this assumption. Experimentally, we have recently shown (Xiang et al., 1992) the permeability of acetamide to be independent of lecithin/decane bilayer thickness when the solvent-filled region of the bilayer was systematically varied, suggesting that the disordered solvent domain contributes negligibly to the overall barrier. It is reasonable that the ordered chain region should constitute the barrier domain as partial chain ordering leads to solute exclusion (White et al., 1981; Marqusee & Dill, 1986; Xiang & Anderson, 1994) and a reduction in free volume (Xiang, 1993). The overall thickness of solvent-free egg lecithin bilayers has been previously determined to be ≈25 Å (Xiang et al., 1992), whereas molecular order parameters measured by deuterium NMR (Stockton & Smith, 1976) indicate that about 72% of the chain segments in the bilayer are in the highly ordered region with $S_{mol} \geq 0.3$. This justifies our selection of 18 Å for the thickness of the proposed barrier domain.

Direct measurement of the relevant barrier domain parameters (i.e., $K_{barrier}$ and $D_{barrier}$) is not readily accomplished for several reasons. First, as pointed out by Diamond and Katz (1974) the zone of *minimum* partition accounts disproportionately for the resistance so that equilibrium determinations of bilayer/water partition coefficients may not reflect the properties of the barrier domain. Determinations of $D_{barrier}$ must take into account the anisotropy of translational diffusion in such highly ordered systems and require knowledge of the translational distribution function of the diffusing species within the bilayer (Moscicki et al., 1993).

We have proposed in a recent statistical mechanical study combined with molecular dynamics simulations (Xiang & Anderson, 1994) that $K_{barrier}$ may be expressed as

$$K_{barrier} = K_{w\to hc}\xi \tag{12}$$

where $K_{w\to hc}$ represents the partition coefficient of solute between an appropriate bulk organic solvent and water. If the organic solvent is chosen to closely mimic the chemical microenvironment of the barrier region, then ξ accounts for the additional work required to create a cavity the size of the solute against the pressure tensor exerted by the surrounding interphase chain molecules. To understand or even properly define the relationship between molecular size and permeability in lipid bilayer systems, therefore, it is essential to identify an appropriate model bulk solvent to correct for the *chemical* selectivity of bilayer transport.

Although alkane solvents are regarded by many as the most suitable model solvents for describing the barrier microenvironment in lipid bilayers (Finkelstein, 1976; Stein, 1986; Walter & Gutknecht, 1986), a recent comparison in the authors' laboratories of functional group contributions to lipid bilayer permeabilities with the effects of the same groups on partitioning in various bulk solvent/water systems using a series of seven solutes with minimal differences in molecular size indicated that the partially unsaturated hydrocarbons 1-hexadecene or 1,9-decadiene more closely resemble the chemical selectivity of egg lecithin bilayers than either hexadecane or octanol. Selectivity coefficients (Eq. 9), s, obtained in that study were 0.85, 0.91, 0.99, and 2.4 for hexadecane, hexadecene, 1,9-decadiene, and octanol, respectively, leading to the selection of 1,9-decadiene as the most suitable model solvent. The results of the present study, displayed in Figs. 4*A-C* and sum-

marized in Table 3, are consistent with our previous evidence favoring 1,9-decadiene or hexadecene over decane as model solvents. While both hexadecene and decadiene partition coefficients gave statistically significant improvements in the fits of log $P_m\delta/K_{w\to hc}$ *vs.* log V using Eq. (8), the difference in fit obtained using 1,9-decadiene partition coefficients rather than hexadecene/water partition coefficients was not significant. Including an additional parameter in the model to allow the selectivity coefficient, *s*, to have a value other than one (Eq. 9) resulted in a substantial improvement in the fit using decane/water partition coefficients and resulted in all three fits becoming virtually identical. In the treatment described by Eq. (9), however, one must consider the value of *s* in addition to the degree of fit in determining which bulk solvent is the most suitable as a model of bilayer chemical selectivity. Again, hexadecene and decadiene are superior to decane.

The possibility that the barrier domain is more polar/polarizable than an alkane solvent can be rationalized by one or both of the following factors: (a) there is a broad distribution of double bonds in the acyl chain molecules within egg lecithin (Fettiplace, Andrews & Haydon, 1971) and since (i) the first double bond in a fatty acid chain is usually located between carbons 9 and 10 with the majority of chains having lengths of 16 or 18 carbons (Fettiplace et al., 1971), (ii) the fluctuation of chain motions along the bilayer normal is on the order of several angstroms (Wiener & White, 1992), and (iii) about 72% of the chain segments in the bilayer are in the highly ordered region with $S_{mol} \geq 0.3$ (Stockton & Smith, 1976), some double bonds in the lipid chains may be present in the effective barrier region; (b) the effective dielectric constant in the barrier domain may be elevated as a result of its proximity to the interface. Unlike in bulk partition experiments where only a very small number of solute molecules are in the vicinity of the interfacial region, a permeant located in the barrier region within a bilayer may be less than 10 Å from the interface. Egg lecithin is a zwitterionic lipid with a carbonyl group dipole moment on the order of 2 D (Smyth, 1955). The electrostatic potential induced by the dipole array of the head groups can reach up to 500 mV in the bilayer interior (McLaughlin, 1977). Substantial penetration of water beyond the head group region of the lipid bilayer can also not be ruled out (Leermakers, Scheutjens & Lyklema, 1983; Egberts & Berendsen, 1988; Nicklas et al., 1991).

The alteration of van der Waals (vdW) attractive interactions between a permeant and the surrounding acyl chains in a bilayer should have only a minor effect on its diffusion coefficient as previous studies have shown that dipole moment does not affect the experimental diffusion coefficients (Chan, 1983). This is also consistent with the rough-hard-sphere model which assumes that molecular translational motions in a liquid are determined primarily by the harsh repulsive parts of the intermolecular forces (Chandler, 1974).

Molecular Volume Dependence of Lipid Bilayer Permeability

According to Eq. (5), lipid bilayer permeability coefficients which have been corrected for an assumed barrier thickness of 18 Å and normalized by $K_{w\to decadiene}$, reflect the product of the diffusion coefficient in the barrier domain, $D_{barrier}$, and the size-dependent part of the partition coefficient, ξ. Figure 5 displays the comparison of the size-dependent components of lipid bilayer transport with diffusion in the bulk hydrocarbon solvent, decane. The disparity in these curves provides additional evidence that the barrier region of egg lecithin/decane bilayers does not resemble the essentially disordered (McIntosh, Simon & MacDonald, 1980; Pope, Walker & Dubro, 1984) decane-filled center of the bilayer, as the permeant size—$P_m\delta/K_{w\to decadiene}$ profiles should have resembled the size-diffusion coefficient profile in bulk decane solvent if the bilayer center were rate limiting. When the size dependence in lipid bilayer permeability was attributed solely to changes in $D_{barrier}$ (i.e., $\xi = 1$; $\alpha = 0$), the *n* values obtained by application of Eq. (6) (Table 3) proved to be extremely sensitive to the model solvent chosen to correct for lipid bilayer barrier/water partitioning, further establishing the importance of choosing the most appropriate model solvent. Using 1,9-decadiene as the reference solvent, an *n* of 1.4 was obtained. The value of *n* in egg lecithin bilayers resembles that for diffusion in polymers when determined in the same manner, where *n* values of 3.8 and 1.1 were found for diffusion in polymethylacrylate and natural rubber, respectively (Lieb & Stein, 1969). Local chain order in amorphous polymers results from the parallel alignment of several neighboring chains to form chain bundles (Pace & Datyner, 1979) and persists over distances of a few tens of angstroms. This observation along with our present results strongly suggest that the transport barrier is located in the ordered hydrocarbon chain region of the bilayer, depicted in Fig. 3.

Free-volume theory for self-diffusion in a liquid of hard spheres, developed by Cohen and Turnbull (Cohen & Turnbull, 1959), suggests that translational diffusion of a solute occurs when statistical redistribution of free volume opens up a void of a critical size in the immediate vicinity of the solute. This theory has been utilized extensively to describe diffusion behavior in polymers (Fujita, 1968; Vrentas et al., 1985 *a,b*). Recent studies have shown, however, that in contrast to the original assumption by Cohen and Turnbull, elementary diffusive displacement may involve only a fraction of the total molecular volume (Mauritz, Storey & George, 1990;

Vrentas & Vrentas, 1990). Taking this into account, diffusion of globular solutes in polymers and simple liquids exhibits size dependencies described by Eq. (6) with n in the range of 2/3–2 (Mauritz et al., 1990). It should be noted, however, that the mathematical form of Eq. (10), which describes the size selectivity for partitioning of globular permeants into the rate-limiting domain of bilayers, resembles the form of the Cohen and Turnbull relationship.

Taking size-dependent partitioning into account by allowing α to vary (Table 3) resulted in a reduction in the value of n to 0.8, close to the value obtained for diffusion in decane, suggesting that the size dependence of the diffusional behavior in bilayers may be less than previously thought (Stein, 1986). From Eqs. (10) and (11), the value α obtained corresponds to a lateral pressure of 300 ± 100 bar in the rate-limiting region in the bilayer. The authors have previously calculated the lateral pressure profile in a model lipid bilayer having a chain length of 16 carbons and surface density of 30 $Å^2$/chain molecule as a function of depth in the bilayer interior by means of molecular dynamics simulation (Xiang & Anderson, 1994). The lateral pressure corresponding to this surface density is 300 bar in the highly ordered barrier region and decreases abruptly in the center of the bilayer. The egg lecithin bilayer has an average chain length of 17.8 carbons and the same surface density as the simulated bilayer (Fettiplace et al., 1971). The good fit of the model and the close agreement between the lateral pressures in the rate-limiting region in the egg lecithin bilayer and the highly ordered chain region in the model bilayer make plausible the hypothesis that a portion of the size dependence in lipid bilayer permeabilities resides in the partitioning component.

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