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

Received: 7 October 1993/Revised: 15 February 1994

Abstract. Permeability coefficients ($P_m$) across planar egg lecithin/decane bilayers and bulk hydrocarbon/water partition coefficients ($K_{w-bc}$) 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 $P_m/K_{w-bc}$ were then ascribed to size-dependent partitioning and/or size-dependent diffusivity. The diffusion coefficient-size dependency was described by $D_{\text{barrier}} = D_o/V^n$. When $n$-decane was used as a reference solvent, the correlation between log $P_m/K_{w-bc}$ 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 ($n = 0.8 \pm 0.3, 1.2 \pm 0.1$ and $1.4 \pm 0.2$, 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, $P_m/K_{w-bc} = D_o \exp(-\alpha V)V^n$. 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 ($D \propto V^{\frac{1}{3}}$, where $n = 2/3$ (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.
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, $K_p$, and the normal component of the diffusion coefficient within the barrier region, $D_n$. 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., hexadecane 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/decan 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, 3H-water, 14C-formic acid, 3H-acetic acid and 14C-acetamide were used. 3H-water, 14C-formic acid and 3H-acetic acid were purchased from ICN Biomedicals (Costa Mesa, CA). 3H-acetamide was synthesized in our laboratory by reaction of 10 mmol 3H-acetic anhydride (New England Nuclear, Boston, MA) with 60 mmol NH$_4$OH (30% in H$_2$O) and the crude product was purified by HPLC. The unlabeled compounds, $\alpha$-substituted $\beta$-toluic acids and 21-[(7-amino-1,7-dioxyheptyl)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 ddf (2',3'-dideoxyinososine) 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 flea 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-