Effect of Pressure on Phospholipid Translocation in Lipid Bilayers

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The organization, location, and transport of lipids and proteins in cells provide a constant background against which many of the essential catalytic events occur.[1] Several physical processes govern the distribution of phospholipids in and among cell membranes. These include lateral diffusion within the bilayer plane, transfer between phospholipid surfaces that are separated by an aqueous compartment, and phospholipid translocation (flip-flop) across a bilayer. Lateral diffusion is fast and relatively independent of the headgroup or acyl chain composition of the phospholipid. Spontaneous transfer between membranes occurs by rate-limiting desorption into the surrounding aqueous phase followed by diffusion-controlled association with the acceptor membrane; this process is a predictable function of the acyl chain lengths of the lipid.[2-7] In contrast, phospholipid translocation across the bilayer is very slow, weakly dependent upon the acyl chain lengths of the lipid and is a function of the identity of the polar headgroup.[8] Hypothetically, the structure of the lipid bilayer should also regulate phospholipid translocation. This can be tested, in part, by substitution of a fluid phospholipid for one that is in the gel or solid phase.[9] However, this involves the use of saturated phospholipids, which are physiologically rare. Another alternative that we have used to study matrix effects is that of elevated hydrostatic pressure.[10] Herein we describe the effects of pressure up to 2 kbar on the rate of flip-flop of 1-octanoyl-2-[9-(1-pyrenyl)nonanoyl]phosphatidylethanolamine (OPNPE).

Briefly, the protocol involves mixing donor single bilayer vesicles composed of OPNPE (5%) and 1-palmitoyl-2-oleoylphosphatidylcholine (95%) with a 20-fold excess of unlabeled vesicles. Transfer kinetics are studied in a pressure vessel, similar to that of Paladini and Weber,[11] which is placed in a thermostatted holder in the sample compartment of a SLM Instruments 8000 fluorometer. The donor and acceptor vesicles were combined in the sample cell and placed in the high-pressure vessel, which was preequilibrated at the experimental temperature. Mixing of the sample, sealing of the pressure vessel, and pressurization results in a dead time of less than 5 min. All transfer reactions were conducted in 100 mM NaCl, 1 mM EDTA, 1 mM NaN_3, and 10 mM 3-(*N*-morpholino)propanesulfonic acid pH 7.4, at 50 °C. The decay of the pyrene excimer fluorescence at 475 nm is composed of an initial immeasurably fast component due to transfer of the pyrenyl lipid from the outer surface of donor vesicles to acceptor vesicles, followed by a slower single exponential that has been identified as rate-limiting translocation across the bilayer, followed by diffusion-controlled transfer to acceptor vesicles.[8] The

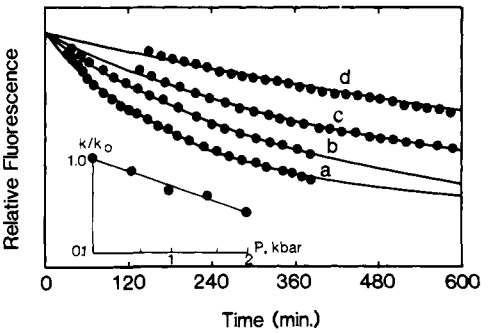

Figure 1. Pressure dependence of phospholipid translocation at 50 °C. Kinetic traces a-d correspond to the decay of the excimer fluorescence of OPNPC at 1, 500, 1000, and 2000 bars. The insert is a semilog plot of the ratio of the rate constants at pressure *P* to those observed at 1 bar. The solid lines are the calculated values fitted to eq 1.

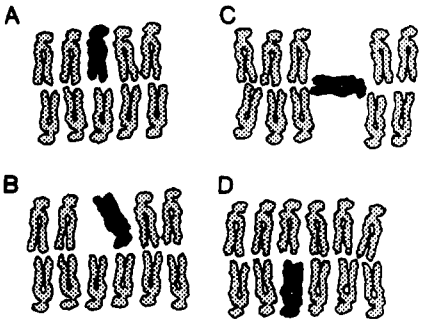

Figure 2. Hypothetical model for phospholipid translocation in lipid bilayers; see text for details.

equation relating reaction velocity to pressure is

$$k = k_0 \exp[-P\Delta V^{\ddagger}/RT] \quad (1)$$

where k and k_0 are the rate constants at pressure P and 1 atm, respectively, and R is the gas constant. At constant temperature, the activation volume, $\Delta V^{\ddagger}$, may be calculated from the slope of a plot of ln k vs. P.

Figure 1 shows representative rate data for the translocation of OPNPE at several pressures. These data show that increasing the pressure produces a decrease in the translocation rate from 0.004 min^{-1} at 1 bar to 0.001 min^{-1} at 2 kbar. When plotted according to eq 1, a linear relationship was found (Figure 1, insert). The slope of this line corresponds to $\Delta V^{\ddagger} = 17 \pm 2$ mL/mol, which means that the volume of the activated complex is greater than that of the initial state. These data allow us to speculate upon the mechanism by which phospholipids spontaneously flip-flop in membranes. Our model, which is shown in Figure 2, is based upon the formation of a transient defect that might occur through statistical fluctuations of phospholipid density in the bilayer plane. A phospholipid that is adjacent to a defect undergoes diffusional rotation toward the bilayer interior (Figure 2B). In most cases the phospholipid rotates back to its original position on the outer leaflet. However, if there is subsequent formation of a defect on the opposite leaflet (Figure 2C), the rotated lipid can readily migrate to that vacancy. If a vacancy is required to accommodate the phospholipid molecule with its long axis rotated parallel to the plane of the bilayer in the rate-limiting step, then one would expect a positive $\Delta V^{\ddagger}$ as we observed. In addition to our experiments, this model is supported by the structural work of others. Both electron spin resonance[12] and fluorescence polarization[13,14] demonstrate that increasing pressure causes increased ordering of molecules located in the hydrocarbon region of the phospholipid bilayer. More importantly, the low angle X-ray scattering data of Braganza and Worcester[15] show that fluid phosphatidylcholine

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bilayers undergo at least a 4% increase in the in-plane packing density with pressurization from 1 bar to 1.9 kbar. This is consistent with a reduction in the lateral compressibility of the lipid and a decrease in the number of defects that are potential conduits for translocation. A model for translocation involving tandomly formed defects on the donor and acceptor leaflets, respectively, is consistent with other data. Small molecules should flip-flop faster than large ones; Homan and Pownall[8] reported that translocation rates decrease with increasing acyl chain length and with the size of the headgroup. Although other effects, such as hydration and headgroup conformation, also affect the rate of translocation, these are probably superimposed on a mechanism involving the tandem defect formation described above. This mechanism could be operative in a native cell membrane wherein the defects are formed at the interface between lipids and integral membrane proteins.

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Hydroxyethylidene (CH_3–C–OH), but Not Ethenol, Tautomerizes to Ethanal

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Although the unimolecular tautomerization of gaseous ethenol to ethanal, CH_2=CH—OH → CH_3CH=O, has been the subject of numerous theoretical investigations,[1-5] no experimental data exist for this reaction, probably due to the inaccessibility of *pure* ethenol. For a century it was suggested as an intermediate in various reactions[6] but was first identified directly in 1973.[7] It has since been prepared by gas-phase pyrolysis[8-12] and identified by using microwave,[8] photoelectron,[10] and mass[11,12] spectra and ionization energy.[9] We report here on the stability and unimolecular reactions of the isomers CH_3—CH=O, CH_2=CH—OH, and CH_3—C—OH (ΔH_f, Figure 1)[13b,9,14a] produced by neutralization[15,16] of the corresponding ions[17,18] and characterized by collisionally activated dissociation (CAD) and product reionization.[15e-h,j]

To obtain neutralization–reionization (NR) mass spectra[15] (Figure 2), mass-selected 10 keV $C_2H_4O^{\bullet+}$ ions are neutralized by Hg (90% transmittance).[15d] Residual ions are deflected, and

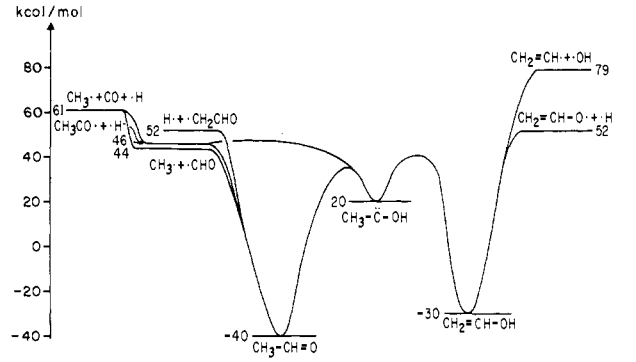

Figure 1. Proposed energy profile for the C_2H_4O neutral tautomers.[9,13,14]

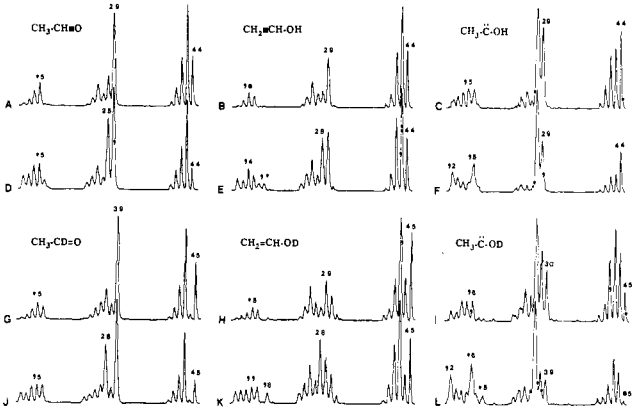

Figure 2. Hg/He NR spectra of CH_3—CH=$O^{\bullet+}$ (A, D), CH_3—CD=$O^{\bullet+}$ (G, J), CH_2=CH—$OH^{\bullet+}$ from cyclobutanol[17] (B, E), CH_2=CH—$OD^{\bullet+}$ from cyclobutanol-O-*d* (H, K), CH_3—C—$OH^{\bullet+}$ from pyruvic acid[18] (C, F), and CH_3—C—$OD^{\bullet+}$ from pyruvic acid-O-*d* (I, L); He transmittances of the neutral beam: 90% (A, B, C, G, H, I) and 30% (D, E, F, J, K, L); *m/z* values beside peaks. Absolute abundances (percent) from identical neutral fluxes: *m/z* 44, A and B, 0.016; C, 0.0095; D, 0.035; E, 0.032; F, 0.029; *m/z* 45, G, 0.012; H, 0.014; I, 0.0033; J, 0.028; K, 0.040; and L, 0.0014. Neutralization yields: CH_3—CH=$O^{\bullet+}$, 9.3%, CH_2=CH—$OH^{\bullet+}$, 6.0%; CH_3—C—$OH^{\bullet+}$, 4.2%, unaffected by isotopic substitution.

the resulting beam of fast neutrals is ionized by collision with He at transmittance values of 90% and 30% (maximum sensitivity), corresponding to ~1 and ~2 collisions, respectively, of the affected species.[19] The additional collision at 30% transmittance can dissociate the primary neutrals prior to reionization.[15j] Consistent with their expected stability, the extent of collisional dissociation of the CH_3—CH=O neutrals and ions is small, relative to scattering; the higher He pressure has little effect on the NR spectra (parts A and D of Figure 2) mainly increasing *m/z* 28, $CO^{\bullet+}$. This should arise from CO or $^{\bullet}CHO$ produced by CAD of neutral, not reionized, CH_3—CH=O (Figure 1), as

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