

Bilayers as Protein Solvents: Role of Bilayer Structure and Elastic Properties

Thomas J. McIntosh¹ and Sidney A. Simon²

¹Department of Cell Biology and ²Department of Neurobiology, Duke University Medical Center, Durham, NC 27710

The perspectives on membrane protein insertion, protein–bilayer interactions, and amino acid side hydrophobicity (*J. Gen. Physiol.* 129:351–377) deal with several key issues in membrane biology. The four informative papers (MacCallum et al., 2007; von Heijne, 2007; White, 2007; Wolfenden, 2007) obtain detailed quantitative data on the interaction of protein amino acid residues with different membrane models. Several types of membrane model systems are used and discussed, including non-polar solvents, such as 1-octanol or cyclohexane, as well as one-component lipid bilayers, such as dioleoylphosphatidylcholine (DOPC) or palmitoyloleoylphosphatidylcholine (POPC). As tabulated by MacCallum et al. (2007) there are appreciable differences among these systems in terms of determining the free energies of transfer of specific amino acid residues from water to nonpolar phases. For example, both White (2007) and Wolfenden (2007) point out possible effects of different amounts of water in the reference phase in terms of measuring the “hydrophobicities” of amino acid residues. Since 1-octanol contains more water and is thus more polar than cyclohexane, one obtains different amino acid partition coefficients between water and these two solvents, particularly for certain residues such as tryptophan (Wolfenden, 2007).

Lipid Bilayers as Solvents

Because lipid bilayers form the core of all biological membranes, bilayers naturally would be expected to have advantages over nonpolar solvents in terms of modeling the hydrophobic interiors of membranes. White (2007) makes the reasonable assumption that the hydrated headgroups of phospholipids can have significant effects on lipid interactions with membrane proteins, most importantly with polar amino acid residues. In this regard, it is gratifying that there is close similarity between amino acid residue hydrophobicity scales as measured with peptides and POPC bilayers (Wimley and White, 1996) compared with protein constructs in translocons in endoplasmic reticulum membranes (von Heijne, 2007). Based on these experiments a liquid-crystalline POPC bilayer appears to provide a good model system for the bilayer of the endoplasmic reticulum. However, the

associations of proteins with bilayers can be regulated by the bilayer’s structural and physical properties that are determined by its composition (Andersen and Koeppe, 2007), which vary for different classes of biological membranes. Thus, the free energy of transfer (ΔG_o) of a peptide from a buffer to a bilayer is not a physical constant (such as for example, the melting point of benzene), but critically depends on the material properties of the bilayer. Just as nonpolar solvents such as 1-octanol and cyclohexane differ in their solvent properties for amino acid residues, so do the bilayers of different biological membranes.

Models for Cell Plasma Membranes

POPC bilayers provide relevant models for the matrix of the endoplasmic reticulum, which is primarily composed of electrically neutral phosphatidylcholines enriched in palmitoyl and oleoyl hydrocarbon chains. However, these one-component POPC bilayers are limited in terms of modeling interactive properties of cell plasma membranes, which, compared with endoplasmic reticulum membranes, contain much higher concentrations of charged phospholipids as well as lipids that modify bilayer structure and material properties, such as phosphatidylethanolamine (PE), sphingomyelin (SM), and cholesterol. Plasma membranes contain functional integral membrane proteins, including ion pumps, channels, receptors, and enzymes, and also interact with important extrinsic proteins and peptides, such as toxins, fusion peptides, antimicrobial peptides, and cytoplasmic proteins. To generalize protein–lipid interactions to the plasma membrane one must take into account its bilayer’s charge distribution, hydrophobic thickness, and material (elastic) properties. Electrostatic interactions between charged bilayers and peptides have been extensively studied and are reasonably well understood (Arbuzova et al., 2000). Therefore, this letter focuses on the less well-appreciated effects of bilayer structural and material properties.

Relevant bilayer structural and material properties include bilayer hydrophobic thickness (h), intrinsic lipid curvature (c_o), area compressibility modulus (K_A), and

Correspondence to T.J. McIntosh: T.McIntosh@cellbio.duke.edu

Abbreviations used in this paper: DOPC, dioleoylphosphatidylcholine; POPC, palmitoyloleoylphosphatidylcholine; SM, sphingomyelin.

bilayer bending modulus (K_B) (Huang, 1986; McIntosh and Simon, 2006; Andersen and Koeppe, 2007). For most bilayers the compressibility and bending moduli are related by $K_B \propto h^2 \cdot K_A$ (Rawicz et al., 2000). The bilayer thickness, which depends on phospholipid chain composition and bilayer cholesterol content, comes into play when the length of a transmembrane protein and h are different (hydrophobic mismatch), and the bilayer adapts with small adjustments in thickness, with the resulting deformation energy depending on c_o , K_A , and K_B (Huang, 1986; Andersen and Koeppe, 2007). It has been shown that a variety of membrane protein activities are regulated by bilayer thickness (Andersen and Koeppe, 2007). The effect on peptide–lipid interactions caused by changes in c_o (which depends on phospholipid headgroup type and hydrocarbon chain unsaturation) has been tested by measuring the free energies of transfer of alamethicin from water to electrically neutral bilayers with different values of c_o obtained by substituting PE for PC and by changing the number of double bonds in the phospholipid hydrocarbon chains (Lewis and Cafiso, 1999). The values of ΔG_o changed by less than 1 kcal/mol with the resulting changes in c_o , or about the same as the energy required to transfer a cysteine residue from the interface to the center of a bilayer (MacCallum et al., 2007).

Much larger changes in ΔG_o have been found with systematic changes in bilayer elasticity caused by the addition of cholesterol. Compared with internal organelle membranes, plasma membranes contain relatively large concentrations of cholesterol (30–40 mol %), which significantly increase K_A (Needham and Nunn, 1990), decrease the depth of water penetration into the bilayer headgroup (Simon et al., 1982), and cause the formation of lateral microdomains or “rafts” (Simons and Ikonen, 1997). As outlined below, each of these cholesterol-induced changes can modify the interactions of proteins with bilayers and thereby impact several of the results in these perspectives.

In terms of K_A , Wolfenden (2007) notes that the energy of transfer of a solute into a solvent involves the cost of making a cavity in the solvent phase, which for a bilayer is directly proportional to K_A (Evans and Skalak, 1979). Since cholesterol markedly increases K_A , this implies that for a given peptide or amino acid, ΔG_o will depend on the concentration of cholesterol in the bilayer. In fact, for the peptide melittin Allende et al. (2003, 2005) showed that the magnitude of ΔG_o is inversely proportional to K_A , such that $\Delta G_o = -7.6$ kcal/mol for DOPC bilayers ($K_A = 265$ dyn/cm), whereas $\Delta G_o = -4.5$ kcal/mol for the much stiffer equimolar SM:cholesterol bilayers ($K_A = 1725$ dyn/cm).

MacCallum et al. (2007) point out the importance of water defects in bilayers in the side chain transfer free energies both to the interface and to the center of DOPC bilayers. For example, charged residues near the

center of the bilayer can be stabilized by large water defects in the bilayer caused by the deformable nature of the bilayer (see Fig. 2 in MacCallum et al., 2007). By decreasing the depth of such water defects, cholesterol should appreciably modify these side chain transfer free energies, although to the best of our knowledge this has not yet been systematically studied.

In plasma membranes, the strong interactions between cholesterol and the saturated hydrocarbon chains of SM cause the formation of lipid rafts. As noted above, raft bilayers composed of SM:cholesterol have different elastic properties than the surrounding matrix (nonraft) bilayer enriched in unsaturated phospholipids such as DOPC, and are also thicker by ~ 7 Å (Gandhavadi et al., 2002). Whereas some membrane proteins are excluded from rafts, others are associated with them, allowing rafts to perform roles in a number of important cellular processes, such as signal transduction and protein trafficking (Simons and Ikonen, 1997; Brown and London, 1998; Simons and Toomre, 2000). Although it is known that some cytoplasmic proteins become associated with rafts when they are acylated (Moffett et al., 2000), the “rules” of partitioning of transmembrane proteins into rafts are currently not well understood and are likely to depend on the effects of bilayer material or elastic properties.

Thus, as documented in the perspectives, physical and chemical properties of specific amino acids are involved in the partitioning of peptides and proteins into the interfacial and hydrocarbon regions of membranes. However, the free energies of partitioning and the depth of the amino acid in the bilayer should also critically depend on bilayer structural and material properties as modified by the bilayer composition.

This work was supported by National Institutes of Health grant GM27278 and by grants from Philip Morris Inc. USA and Philip Morris International.

Olaf S. Andersen served as editor.

REFERENCES

- Allende, D., A. Vidal, S.A. Simon, and T.J. McIntosh. 2003. Bilayer interfacial properties modulate the binding of amphipathic peptides. *Chem. Phys. Lipids*. 122:65–76.
- Allende, D., S.A. Simon, and T.J. McIntosh. 2005. Melittin-induced bilayer leakage depends on lipid material properties: evidence for toroidal pores. *Biophys. J.* 88:1828–1837.
- Andersen, O.S., and R.E. Koeppe. 2007. Bilayer thickness and membrane protein function: an energetic perspective. *Annu. Rev. Biophys. Biomol. Struct.* 36:107–130.
- Arbuzova, A., L. Wang, J. Wang, G. Hangyas-Mihalayne, D. Murray, B. Honig, and S. McLaughlin. 2000. Membrane binding of peptides containing both basic and aromatic residues. Experimental studies with peptides corresponding to the scaffolding region of the caveolin and the effector region of MARCKS. *Biochemistry*. 39:10330–10339.
- Brown, D.A., and E. London. 1998. Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* 14:111–136.

- Evans, E.A., and R. Skalak. 1979. Mechanics and Thermodynamics of Biomembranes: Part 2. CRC Critical Reviews in Bioengineering. CRC Press, Boca Raton, FL. 331–418.
- Gandhavadi, M., D. Allende, A. Vidal, S.A. Simon, and T.J. McIntosh. 2002. Structure, composition, and peptide binding properties of detergent soluble bilayers and detergent resistant rafts. *Biophys. J.* 82:1469–1482.
- Huang, H.W. 1986. Deformation free energy of bilayer membrane and its effect on gramicidin channel lifetime. *Biophys. J.* 50:1061–1070.
- Lewis, J.R., and D.S. Cafiso. 1999. Correlation of the free energy of a channel-forming voltage-gated peptide and the spontaneous curvature of bilayer lipids. *Biochemistry.* 38:5932–5938.
- MacCallum, J.L., W.F.D. Bennett, and D.P. Tieleman. 2007. Partitioning of amino acid side chains into lipid bilayers: results from computer simulations and comparison to experiment. *J. Gen. Physiol.* 129:371–377.
- McIntosh, T.J., and S.A. Simon. 2006. Roles of bilayer material properties in function and distribution of membrane proteins. *Annu. Rev. Biophys. Biomol. Struct.* 35:177–198.
- Moffett, S., D.A. Brown, and M.E. Linder. 2000. Lipid-dependent targeting of G proteins into rafts. *J. Biol. Chem.* 275:2191–2198.
- Needham, D., and R.S. Nunn. 1990. Elastic deformation and failure of lipid bilayer membranes containing cholesterol. *Biophys. J.* 58:997–1009.
- Rawicz, W., K.C. Olbrich, T. McIntosh, D. Needham, and E. Evans. 2000. Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophys. J.* 79:328–339.
- Simon, S.A., T.J. McIntosh, and R. Latorre. 1982. Influence of cholesterol on water penetration into bilayers. *Science.* 216:65–67.
- Simons, K., and E. Ikonen. 1997. Functional rafts in cell membranes. *Nature.* 387:569–572.
- Simons, K., and D. Toomre. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* 1:31–39.
- von Heijne, G. 2007. Formation of transmembrane helices in vivo—is hydrophobicity all that matters? *J. Gen. Physiol.* 129:353–356.
- White, S.H. 2007. Membrane protein insertion: the biology-physics nexus. *J. Gen. Physiol.* 129:363–369.
- Wimley, W.C., and S.H. White. 1996. Experimentally determined hydrophobicity scale for proteins at membrane interfaces. *Nat. Struct. Biol.* 3:842–848.
- Wolfenden, R. 2007. Transmembrane and globular proteins. *J. Gen. Physiol.* 129:357–362.