Dynamic Heterogeneities of the Lipid Matrix of Biological Membranes and Associated Protein Function

Rodney L. Biltonen

Abstract

This report will focus on the physical basis for lateral heterogeneity in the lipid matrix of the bilayer membrane and how the associated properties can play a role in the function of membrane-associated proteins. The propensity for lateral heterogeneity can provide sites of lateral density fluctuations, enhance both lipid and protein domain formation and lead to local and global structural changes in the bilayer. The activation of phospholipase A_2 and protein kinase C will be used as two examples demonstrating the correlation between lateral heterogeneity and protein function.

Rodney L. Biltonen
Departments of Biochemistry and Pharmacology
University of Virginia Health Sciences Center
Charlottesville, VA 22908, USA
e-mail: rlb1t@uva.pcmail.virginia.edu

Biol. Skr. Dan. Vid. Selsk. 1998, **49**:41–45

The lipid matrix of the biological membrane is a heterogenous mixture of lipids existing in varying degrees of individual disorder, the distribution of which is continuously fluctuating with time. Both compositional fluctuations and thermal fluctuations can lead to local and global structural changes. In addition, the composition of the membrane can be altered by the action of enzymes such as the lipases producing fatty acids, lysophospholipids and diacylglycerols. These lipid metabolites can act as protein effectors and also produce alterations in lipid distribution and membrane structure. It is these heterogenieties in composition and structure and alterations therein which could play major roles in the activation and modulation of membrane-associated protein function (Biltonen, 1990; Kinnunen, 1991; Mouritsen and Biltonen, 1993; Mouritsen and Jørgensen, 1994).

In single component systems, fluctuations in the degree of order or structure of any individual lipid are dictated by the energies and degeneracies of the accessible structural states and, to the

first approximation, the various nearest-neighbor interactions. Long range correlations in structure may exist depending upon the differences in the nearest-neighbor interactions among the various accessible states. For example, the parameter determining the extent of long-range correlation in a system in which a lipid can exist in only one of two states is

$$W = E_{a,b} - 1/2(E_{a,a} + E_{b,b})$$

where $E_{i,j}$ is the interaction energy between two neighboring lipids in states i, j. If $W = 0$ the distribution of lipid in the two structural states over the membrane lattice will be random, whereas if $W = \infty$ the lattice of any single membrane will be found completely in either state a or b. Between these two extremes the membrane lattice will exist in some nonrandom lipid organization producing a distribution of clusters of varying size. The cluster distribution will depend upon the intrinsic probabilities of the lipid existing in a or b and the magnitude of the interaction term, W (Heimburg and Biltonen, 1996; Jerala et al., 1996).

The probability of the lipid existing in state a or b in a single component system is a function of the free energy change associated with a structural transition. In a two component system this probability is also related to the composition of the system. Even if the matrix is in one phase (e.g. gel) compositional heterogeneities will exist. In this case W will be defined by the interactions between the chemically distinct species, defined by such aspects of the system as acyl-chain mismatch (Mouritsen and Jørgensen, 1994). In more complex systems, the situation becomes more difficult to define, but the basic features remain: The bilayer will possess heterogeneities in structure and composition which could result in distinct cluster or domain formation which, in turn, could strongly influence membrane-associated protein function.

Domain formation within the lipid matrix of the biological membrane can influence function in several ways. For example, density fluctuations at the interface between distinct domains could ease the penetration of peptide sequences into the hydrophobic interior of the membrane or for the lipid hydrolases, these regions could be the location for optimal interaction between the enzyme and substrate. The existence of clusters could enhance the probability of protein aggregation on the lipid surface by promoting regions of high local concentrations of protein (Sperotto and Mouritsen, 1993; Tocanne et al., 1994; Heimburg and Biltonen, 1996). Here it should be noted that the clusters or domains do not need to preexist, but protein localization will occur if only there is a propensity for lipid domain existence. Proteins can induce domain formations via thermodynamic coupling between lipid-lipid interactions and protein-lipid interactions. Finally, lipid domains and protein/lipid domains can alter the kinetics of processes requiring diffusion in the plane of the membrane (Thompson et al., 1995).

If domain formation, rather than the existence of a particular phase, is functionally important then it is expected that an extremum in certain functional behaviors (e.g. enzyme activation) will be observed in regions of compositional or temperature variation where domain coexistence is maximal. This is indeed observed for the activation of phospholipase A_2 as a function of temperature (Hønger et al., 1996) and the activation of protein kinase C as a function of diacylglycerol mole fraction (Dibble et al., 1996). Although the two examples to be discussed are related to lipid systems in the gel state or the gel-liquid coexistence region, regions of compositional heterogeneity in binary mixtures in the liquid state have been suggested by Pedersen et al. (1996) using fluorescence energy transfer and Lehtonen et al. (1996) using pyrene excimer fluorescence.

Phospholipase A_2 exhibits a long lag period until maximal activity is observed when large unilamellar vesicles composed of saturated phosphatidylcholine vesicles are used as the substrate. This lag period is a function of temperature, substrate and calcium concentration. This lag period exhibits a minimum very close to the gel-liquid phase transition temperature, a temperature at which phase coexistence occurs (Romero et al., 1987) . Recently, Hønger et al. (1996) using a ten-state model in a Monte Carlo simulation, demonstrated that the inverse of the lag period was correlated with the calculated amount of interfacial area between gel and liquid domains.

It has previously been shown that the onset of rapid hydrolysis occurred when a precise amount of reaction product (fatty acid and lysophospholipid) were produced (Bell and Biltonen, 1992). Burack, et al. (1995) showed that this onset was not the result of enhanced binding because of the presence of the negatively charged fatty acid. Rather, they demonstrated with a pyrene-labelled fatty acid that lateral phase separation of the reaction products from the bulk lipid occurred at that point (Burack and Biltonen, 1994). This compositional demixing then leads to a change in morphology of the aggregated substrate (Hønger et al., 1995; Burack et al., 1997). While the details of this activation process have remained elusive, these studies clearly show that activation is intimately related to the structural and compositional heterogeneity of the lipid bilayer.

Protein kinase C is activated on membrane surfaces and requires the presence of phosphatidylserine and diacylglycerol. The negatively-charged lipid enhances binding of the protein to the surface and the diacylglycerol (Nakamura and Nishizuka, 1994; Newton and Keranen, 1994) is thought to be a specific activator, the mechanism of which is unclear. Working with the hypothesis that the structural aspects of the membrane (Sando et al., 1992; Sando and Chertihin, 1996), specifically lipid domain formation, could play a key role in the activation process, we undertook a study of a ternary system containing these components (Dibble et al., 1996; Hinderliter et al., 1997). The pseudo phase diagram of a 1:1 mixture of phosphatidylcholine/phosphatidylserine as a function of the concentration of various diacylglycerols was determined with differential scanning calorimetry. The results of these experiments indicated that a 1:1:1 compound formation occurred and that in the range of about 0.1 to 0.35 mole-fraction of the diacylglycerol two phases, one rich in diacylglycerol and one poor in diacylglycerol, coexisted in the gel state. The formation of the compound was verified by FTIR experiments which demonstrated that all three components (including the diacylglycerol which melts at a low temperature) melt concurrently in the vicinity of 25°C when the mole-fraction of diacylglycerol was sufficiently high.

What was most significant was that the activity of protein kinase C increased as the system in the gel state entered the putative compositional phase coexistence region, achieving a maximum near mole-fraction 0.2 and then falling as the diacylglycerol component was further increased. This observed increase in activity is consistent with domain formation playing a role in activation and could be related to lipid-induced clustering of the protein or the existence of domain interfaces. It is interesting to note that Glaser and coworkers have recently shown by fluorescence microscopy (Yang and Glaser, 1995; Glaser et al., 1996) that lipid and substrate clustering appear to be related to protein kinase C activation (see also McLaughlin, this volume.)

One aspect of compositional heterogeneity or phase separation that deserves special mention is that strong coupling between lateral phase separation or compositional differences in the two planes of the bilayer and membrane mechanical stress can exist. Such stress can lead to strong fluctuations in the plane normal to the membrane surface and could be important in many membrane processes as has been pointed out by Sackmann and Feder (1995). Lundbæk and Andersen (1994) have also suggested that changes in membrane deformation energy caused by certain membrane-modifying compounds such as lysophospholipids can modulate membrane channel function. In extreme cases it could lead to major changes in membrane morphology (Burack and Biltonen, 1994; Hønger et al., 1995; Burack et al., 1997). Finally, it should be emphasized that fluctuations in the structure of the lipid matrix of the biological membrane can occur over a very wide time scale. While some local fluctuations may be very rapid (nanoseconds to microseconds), relaxations in cooperative structure may occur on the time scale of milliseconds to hours (van Osdol et al., 1991; Biltonen and Ye, 1994; Jørgensen et al., 1996). The dynamic coupling of the bilayer fluctuations to protein processes is an area that is yet to be investigated in much detail. In any case, it seems clear that lipid domain formation is an important feature of membrane structure and that the existence of distinct domains and their time-dependent

variation may play significant roles in cellular function.

***

The work reported herein was supported by the National Science Foundation, The National Institutes of Health and The Danish Technical Research Council and is the result of many extensive collaborations including those with Drs. Thomas Hønger, Kent Jørgensen and Ole G. Mouritsen of the Technical University of Denmark and Drs. John D. Bell, W. Richard Burack, Andrew R. Dibble, Martha Gadd, Anne K. Hinderliter, Guilleromo Romero, Julianne J. Sando, Kim K. Thompson and Thomas E. Thompson of the University of Virginia. I am deeply indebted to them for their experimental contributions and many useful discussions.

References

Bell, J.D., and R.L. Biltonen. 1992. Molecular Details of the Activation of Soluble Phospholipase A_2 by Lipid Bilayer Substrates: Comparison of Computer Simulations with Experimental Results. *J. Biol. Chem.* 267: 11046-11056.

Biltonen, R.L. 1990. A Statistical Thermodynamic View of Cooperative Structural Changes in Phospholipid Bilayer Membranes: Their Potential role in Biological Function. *J. Chem. Therm.* 22:1-19.

Biltonen, R.L. and Q. Ye. 1994. Differential Scanning and dynamic calorimetric studies of cooperative phase transitions in phospholipid bilayer membranes. *Subcellular Biochemistry* 23:121-160.

Burack, W.R. and R.L. Biltonen. 1994. Lipid Bilayer Heterogeneities and Modulation of Phospholipase A_2 Activity. *Chem. Phys. Lipids* 73:209-222.

Burack, W.R., M.E. Gadd, and R.L. Biltonen. 1995. Modulation of Phospholipase A_2: Identification of an Inactive Membrane-bound State. *Biochemistry* 34:14819-14828.

Burack, W.R., A.R.G. Dibble, M.M. Allieta, and R.L. Biltonen. 1997. Changes in Vesicle Morphology Induced by Lateral Phase Separation Modulate Phospholipase A_2 Activity. *Biochemistry* 36:10551-10557.

Dibble, A.R.G., A. Hinderliter, J.J. Sando, and R.L. Biltonen. 1996. Lipid lateral heterogeneity within phosphatidylcholine/phosphotidylserine/diacylglycerol vesicles and its influence on protein kinase C activation. *Biophysical J.* 71:1877-1890.

Glaser, M., S. Wanaski, C.A. Buser, V. Boguslavsky, W. Rashidzada, A. Morris, M. Rebecchi, S.F. Scarlata, L.W. Runnels, G.D. Prestwich, J. Chen, A. Aderem, J. Ahn, and S. McLaughlin. 1996. Myristoylated alenine-rich C kinase substrate (MARCKS) produces reversible inhibition of phospholipase C by sequestering phosphatidylinositol 4,5-bisphosphate in lateral domains. *J. Biol. Chem.* 271:26187-26193.

Heimburg, T., and R.L. Biltonen. 1996. A Monte-Carlo Simulation Study of Protein Induced-Heat Capacity Changes and Lipid-Induced Protein Clustering. *Biophysical J.* 70:84-96.

Hinderliter, A.K., A. Dibble, R.L. Biltonen, and J.J. Sando. 1997. Activation of Protein Kinase C by Coexisting Diacylglycerol- enriched and Diacylglycerol-Poor Lipid Domains. *Biochemistry* 36:6141-6148.

Hønger, T.E.R., A.R.G. Dibble, W.R. Burack, M.M. Allietta, and R.L. Biltonen. 1995. Vesicle Morphology Modulates Phospholipase A_2 Activity. *Biophys. J.* 68: A218.

Hønger, T., K. Jørgensen, R.L. Biltonen, and O.G. Mouritsen. 1996. Correlation between phospholipase A_2 activity and dynamic lipid bilayer micro-heterogeneity. *Biochemistry* 35:9003-9006.

Jerala, R., P.F.F. Almeida, and R.L. Biltonen. 1996. Simulation of the Gel-Fluid Transition in a Membrane Composed of Lipids with Two connected Acyl Chains: Application of a Dimer-move Step. *Biophysical Journal* 71:609-615.

Jørgensen, K., A. Klinger, M. Braiman, and R.L. Biltonen. 1996. Slow non-equilibrium dynamical rearrangement of the lateral structure of a lipid membrane. *J. Phys. Chem.* 100:2766-2769.

Kinnunen, P.K.J. 1991. On the Principles of Functional Ordering in Biological Membranes. *Chem. Phys. Lipids* 57:375-399.

Lehtonen, J.Y.A., J.M. Holopainen, and P.K.J. Kinnunen. 1996. Evidence for the Formation of Microdomains in Liquid Crystalline Large Unilamellar Vesicles Caused by Hydrophobic Mismatch of the Constituent Phospholipids. *Biophys. J.* 70:1753-1760.

Lundbæk, J.A. and O.S. Andersen. 1994. Lysophospholipids Modulate Channel Function by Altering the Mechanical Properties of Lipid Bilayers. *J. Gen. Physiol.* 104:645-673.

Mouritsen, O.G., and R.L. Biltonen. 1993. Protein-Lipid Interactions and Membrane Heterogeneity. The New Comprehensive Biochemistry: Protein-Lipid Interactions (A. Neuberger and L.L.M. Van Dennen, eds.) *Elsevier.* pp. 1-39.

Mouritsen, O.G., and K. Jørgensen. 1994. Dynamical Order and disorder in lipid bilayers. *Chem. Phys. Lipids* 73:3-25.

Nakamura, S.-I., and Y. Nishizuka. 1994. Lipid Mediators and Protein Kinase C Activation for the Intracellular Signaling Network. *J. Biochem.* 115:1029-1034.

Newton, A.C., and L.M. Keranen. 1994. Phosphatidyl-L-Serine is Necessary for Protein Kinase C s High-Affinity Interaction with Diacylglycerol-Containing Membranes. *Biochemistry* 33:6651-6658.

Pedersen, S., K. Jørgensen, T.R. Baekmark, and O.G. Mouritsen. 1996. Indirect Evidence for Lipid Domain Formation in the Transition Region of Phospholipid Bilayers for Two-Probe Fluorescence Energy Transfer. *Biophys. J.* 71:554-560.

Romero, G., K. Thompson, and R.L. Biltonen. 1987. The Activation of Procine Pancreatic Phospholipase A_2 by Dipalmitoylphosphatidylcholine Large Unilamellar Vesicles. *J. Biol. Chem.* 262: 13476-13480.

Sackmann, E., and T. Feder. 1995. Budding, fusion and domain formation in mixed lipid vesicles induced by later phase separation and macromolecular condensation. *Mol. Memb. Biol.* 30:4516:4521.

Sando, J.J., M.C. Maurer, E.J. Bolen, and C.M. Grisham. 1992. Role of Cofactors in Protein Kinase C Activation. *Cell. Signalling* 4:595-609.

Sando, J.J. and O.I. Chertihin. 1996. Activation of Protein Kinase C by Lysophosphatidic Acid: Dependence on Composition of Phospholipid Vesicles. *Biochem. J.* 317:583:588.

Sperotto, M.M., and O.G. Mouritsen. 1993. Lipid Enrichment and Selectivity of Integral Membrane Proteins in Two Component Lipid Bilayers. *Eur. Biophys. J.* 22:323-328.

Thompson, T.E., M.B. Sankaram, R.L. Biltonen, D. Marsh, and W.L.C. Vaz. 1995. Effects of domain structure on in-plane reactions and interactions. *Mol. Memb. Biol.* 12:157-162.

Tocanne, J.-F., L. Dupou-Cezanne, A. Lopez, E. Perochon, B. Piknova, V. Schram, J.-F. Tournier, and M. Welby. 1994. Lipid Domains and Lipid/Protein Interactions in Biological Membranes. *Chem. Phys. Lipids* 73:139-158.

van Osdol, W., Q. Ye, M.J. Johnson, and R.L. Biltonen. 1991. The Effect of Chainlength and Vesicle Size on the Dynamics of the Gel-Liquid Crystalline Transition of Lecithin Bilayers. *Biophysical J.* 59:775-785.

Yang, L. and M. Glaser. 1995. Membrane Domains Containing Phosphatidylserine and Substrate can be Important for the Activation of Protein Kinase C. *Biochemistry* 34:1500-1506.