Connectivity of membrane domains

Derek Marsh
Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany

Organisms exist by virtue of the specific compartmentation afforded by the plasma membrane and the membranes of the intracellular organelles. A less clear issue is the extent to which possible two-dimensional compartmentation arising from in-plane membrane domains is essential to the life of the cell (cf. 1). Domain formation, corresponding to regions of lateral phase separation, has been visualized in bilayer membranes composed of binary lipid mixtures (originally by McConnell and associates). The potential functional advantages arising from the enhanced lateral compressibility of membranes in a state of lateral phase separation have long been recognized. Only recently, however, has attention turned again to membrane domains as a means of compartmentation, and more importantly as channels of communication, within the plane of the membrane (2). Vaz, Thompson, and co-workers (3–5) have pioneered studies which have illustrated communication between coalescing domains in a rather direct way. Conceptually the experiments are rather simple, but the results are most striking nonetheless. For many years, photobleaching a given area of membrane in which fluorophores are homogeneously incorporated has been used to determine translational diffusion coefficients from the recovery of fluorescently labelled species into the region bleached. The question now asked is: what happens when such measurements are performed with a fluorescently labelled lipid incorporated in binary lipid membranes that are in a region of lateral phase separation? The answer is that the fluorescence recoveries remain extremely low (even though the fluorophore is generally restricted to domains of fluid lipid), until the temperature is raised to such a point that the various fluid membrane domains connect with one another to form a continuous domain of dimensions comparable to that of the bleached membrane area (typically ≈ 1 μm). At this point of connectivity, the fluorescence recovery increases abruptly to 100%. The point of connectivity is found to display a fascinating dependence on the lipid composition of the binary mixture. In some cases, connectivity of fluid domains is found to occur close to the *fluidus* phase boundary (3), i.e., only when most of the lipid is converted to the fluid phase, whereas in other cases domain connectivity is found close to the *solidus* phase boundary (5) when only a small proportion of the lipid is in the fluid phase. Clearly, the geometry of the domains must be very different in these two cases: one is isometric whereas the other is reticular. Such results have also stimulated the development of other methods for determining domain sizes (6). In the current issue of this journal, Almeida et al. have extended the previous studies to ternary lipid mixtures which include the ubiquitous mammalian plasma membrane component, cholesterol. This is significant for several reasons: not only is the compositional complexity of the membrane increased, but also cholesterol is known to remove the cooperativity of the sharp phase transitions associated with single phospholipid species. Therefore, the properties of such mixtures may be representative not only of cholesterol-containing plasma membranes, but also of the non-cooperative behavior of the complex lipid mixtures found in other membranes. Additionally, the connectivity data have been interpreted by Almeida et al. in terms of general results from percolation theory. This opens up the possibility of analyzing other types of membrane domain formation, for which the lipid mixtures serve as a well defined test system. With this, domain connectivity in membranes can really be considered to have come of age. The wheel has almost turned full circle, since it is now possible to consider again, as previously suggested (7), whether the incomplete recoveries with fluorescently labelled proteins, that are normally found after photobleaching cell membranes, are features of another form of domain disconnection. Combined with measurements of the local protein diffusion coefficients (8), the answer to this question may be in sight.

Received for publication and in final form 13 November 1992.

REFERENCES

1. Marsh, D. 1992. Role of lipids in membrane structures. *Curr. Opin. Struct. Biol.* 2:497–502.
2. Thompson, T. E., M. B. Sankaram, and R. L. Biltonen. 1992. Biological membrane domains: functional significance. *Comm. Mol. Cell Biophys.* 8:1–5.
3. Vaz, W. L. C., E. C. C. Melo, and T. E. Thompson. 1989. Translational diffusion and fluid domain connectivity in a two-component, two-phase phospholipid bilayer. *Biophys. J.* 56:869–876.
4. Vaz, W. L. C., E. C. C. Melo, and T. E. Thompson. 1990. Fluid-phase connectivity in an isomorphous, two-component, two-phase phosphatidylcholine bilayer. *Biophys. J.* 58:273–275.
5. Bultmann, T., W. L. C. Vaz, E. C. C. Melo, R. B. Sisk, and T. E.

Thompson. 1991. Fluid-phase connectivity and translational diffusion in a eutectic, two-component, two-phase phosphatidylcholine bilayer. *Biochemistry.* 30:5573–5579.

6. Sankaram, M. B., D. Marsh, and T. E. Thompson. 1992. Determination of fluid and gel domain sizes in two-component, two-phase lipid bilayers. An electron spin resonance spin label study. *Biophys. J.* 63:340–349.
7. Edidin, M. 1990. The super-ordered fluid: a domain model of cell surface membranes. *In* Biophysics of the Cell Surface. R. Glaser and D. Gingell, editors. Springer-Verlag, Berlin. 51–59.
8. Esmann, M., and D. Marsh. 1992. Local translational diffusion rates of membranous Na^+, K^+-ATPase measured by saturation transfer ESR spectroscopy. *Proc. Natl. Acad. Sci. USA.* 89:7606–7609.