Chapter 4

Lipid Composition of Membrane Domains

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Abstract
The isolation of subfractions of cell membranes on the basis of their solubility in non-ionic detergents has led to the discovery of lipid domain structure in membranes. Detergents used for this purpose include Triton, Brij, Lubrol and CHAPS. Different lipid constituents are known to resist solubilization by different detergents and the resulting fractions may associate with different membrane proteins. In general, the detergent-resistant membrane fractions tend to be dominated by saturated molecular species of sphingomyelin and phosphatidylcholine and invariably include significant proportions of cholesterol. The lipid composition is consistent with formation of liquid-ordered phases. The present evidence favours a model in which the lateral segregation of membrane proteins takes place on the basis of their affinity for liquid-ordered lipid domains within the membrane.

1. INTRODUCTION
The existence of domains in the surface membrane of cells has been postulated by observing the motion of constituent molecules. Much of the evidence demonstrating constraints on motion include single particle tracking of
colloidal gold labelled molecules on the surface of living cells (Daumas et al., 2003), the fluorescence observed on partitioning of fluorescent probes into restricted domains (Gousset et al., 2002), single dye tracing (Schutz et al., 2000; Dietrich et al., 2002), fluorescence microscopy (Radhakrishnan et al., 2000; Dietrich et al., 2001a,b; Dietrich et al., 2001; Anderson and McConnell 2002) and fluorescence quenching of membrane probes (Wang and Silvius 2000, 2001).

The creation of domains of restricted motion where the properties are distinct from domains where the motion of constituents is relatively fluid implies that the composition in lateral regions are different. While this is obvious in the case of specialized membrane domains like intercellular junctions and the like, where particular membrane proteins dominate the organisation of the constituents, the detection of membrane rafts is not so clear cut. In raft domains, it is suggested that the interaction between membrane lipids is responsible for segregation of the constituents so it might be anticipated that the lipid composition would be characteristic of such fractions.

The major challenge to research in this area has been to isolate membrane domains and to compare the respective composition of the motionally restricted regions with those of the fluid domains. The use of non-ionic detergents like Triton, Brij, Lubrol and 3-[3-cholamidopropyl]dimethylammonio]-1-propyl sulphonate (CHAPS) to separate soluble and insoluble fractions of membranes has provided an operational basis for the isolation of rafts which resist solubilization (detergent-resistant membranes; DRM). The influence of the detergent in promoting domain formation by segregating components present in the parent membrane remains somewhat conjectural. This chapter will examine the lipid composition of detergent-resistant membrane preparations examine the evidence as to whether detergents segregate lipids into rafts and the extent to which these detergent-resistant membrane fractions resemble domains present in the original membrane.

2. FRACTIONATION OF MEMBRANE DOMAINS

Kirkpatrick and colleagues (Kirkpatrick et al., 1974) were amongst the first to report selective solubilization of membrane lipids by treatment with Triton X-100. They showed that membrane proteins and lipids were solubilized in human erythrocyte membranes with increasing concentrations of detergent up to 5 mM. The insoluble fractions under these conditions were found to preferentially retain sphingomyelin and cholesterol.

Since then many studies have shown that treatment of a range of cell membranes with mild detergents at low temperatures (0–4°C) results in selective solubilization of the structure (Ahmed et al., 1997; Schroeder et al., 1998; Janes et al., 2000; Xu and London 2000; Li et al., 2001; Wang et al., 2001; Xu et al., 2001).