LIPID LATERAL PHASE SEPARATIONS

SPIN LABEL AND FREEZE-FRACTURE ELECTRON MICROSCOPY STUDIES

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The current concept of many biological membranes is one of structures having considerable potential for component mobility in two dimensions. Hence long-range order in membranes and methods of disturbing or maintaining it have become research topics of some interest.

There is an increasing body of data indicating that membrane components (both protein and lipid) are not simply randomly mixed but that they interact in such a way as to segregate or pool into specialized regions. Fully hydrated lipids can undergo a process analogous to melting in solids while maintaining their bilayer array, and this leads to a possible mechanism for generating a mosaic distribution of some components in a membrane. The process in question is the cooperative transition of lipid acyl chains from a rigid (gel) state to a fluid (liquid crystal) state (see reference 1 and references contained therein). This phenomenon has been investigated in a wide variety of systems ranging from simple, one component lipid bilayers to intact, viable organisms. When two pure solids having different melting points are homogeneously mixed, the new melting behavior is in general distinct from that of either original component and, for a given pressure, can be described by a temperature vs. composition phase diagram. The important point is that such mixtures can exhibit phase separations, i.e. coexistence of distinct regions of different composition and properties (typically these regions will not contain either component in pure form but will be selectively enriched in one or the other). In the case of membranes such separations are two-dimensional.

Evidence has recently been presented that lipid lateral phase separations can affect protein distribution in model systems (2) and intact organisms (see reference 3 and references contained therein). However it is not yet known how (or indeed whether) organisms actually *make use* of this phenomenon for maintaining or altering membrane structure and function.

The small, inert spin label, 2,2,6,6,-tetramethylpiperidine-1-oxyl (Tempo), has proven useful in monitoring fluid-rigid transitions (and hence phase separation phenomena) in membranes (4) and lipid mixtures (5). It partitions between the aqueous

phase and the lipid hydrophobic regions and the high field resonance of its EPR spectrum is readily analyzed into separate components from each of these two spin label pools. In the past, workers have found it convenient to use the peak height of the lipid pool component and that of the aqueous pool component as first approximations to the amount of spin label in each. It is very easy to measure the ratio of the lipid peak height to the sum of both peak heights as a function of, say, temperature. Since Tempo is preferentially excluded from rigid lipid regions, the temperature-induced changes in this ratio are a direct measure of the fluid-rigid lipid interconversion. It is possible to refine the analysis, for instance by taking into account underlying ^{13}C shoulders and linewidth differences between the two spin-label pools. But in general this is unnecessary since the observable of interest is a sharp change in the ratio as a function of temperature and not the absolute value. The above-mentioned peak height ratio is the so-called "Tempo parameter" (5). Break points in plots of this ratio vs. temperature have been used to locate phase transitions in pure hydrated lipids and the onset or completion of phase separations in lipid mixtures and cell membranes (3–6). In binary lipid systems phase diagrams have been constructed from such data (5, 6).

As mentioned above, the usefulness of the spin label, Tempo, stems from its sensitivity to processes leading to a physical rigidification of the lipid in a membrane. This nonspecific mechanism has led to its employment in a wide range of systems. However for a given system some other form of spin label may be more advantageous. For instance, in the work of Ohnishi and Ito on calcium-induced phase separations (7), exchange broadening of a spin-labeled phospholipid spectrum proved to be a sensitive indicator.

It has also been possible to study problems involving component distribution in membranes by freeze-fracture electron microscopy. Using this sample preparation technique one is able to examine extensive regions of lipid bilayers and biological membranes under different conditions of temperature, pH, etc. The distribution of proteins can be followed directly if they give rise to intramembranous particles in the otherwise smooth fracture faces (8). Furthermore protein fragments substantial enough to cast a heavy metal shadow under the sample preparation conditions involved show up as particles on membrane etch faces (the cytoplasmic and external membrane surfaces). This latter fact has been used in protein distribution studies but usually it is necessary to prelabel with a bulky group such as ferritin.

The problem of lipid distribution is less straightforward since bilayer regions of biological membranes are generally smooth and featureless in freeze-fracture electron micrographs. Nevertheless in certain cases lipids display freeze-fracture patterns characteristic of whether they are in the fluid or the rigid state. For instance, a number of phosphatidylcholines (and simple mixtures thereof) have characteristic orderly, parallel ripple patterns on fracture and etch faces when in the gel state (9, 10). The fluid (liquid crystal) state is distinguishable in that such regions appear almost smooth or have a highly disordered pattern. In several cases this has provided an opportunity to independently check spin label-derived conclusions regarding lipid lateral phase separations. For instance in the case of lipid bilayer vesicles formed

from binary mixtures of dimyristoyl and distearoyl phosphatidylcholines or of dielaidoyl and dipalmitoyl phosphatidylcholines, it is possible to vary temperature and/or composition to produce *coexisting* fluid and rigid domains. Freeze-fracture electron micrographs of such systems clearly show these lipid phase separations, and the relative amounts of each type of domain for a given set of conditions is in good agreement with the spin label-derived phase diagrams (3, 6, 11). The domains seen in these studies were relatively large (dimensions on the order of 1,000 Å).

The effect of such lipid lateral phase separations on the distribution of the glycoprotein, glycophorin, from human erythrocytes and on that of the (Mg^{2+} + Ca^{2+}) ATPase from rabbit sarcoplasmic reticulum has been tested in various lipids including the two binary mixtures mentioned above (2, 12). These systems were prepared by dissolving lipid mixture plus protein in one of several buffered detergents and then dialyzing away the detergent to get lipid vesicles whose walls contained the protein in question. Freeze-fracture electron micrographs show the protein-related intramembranous particles to be consistently forced out of rigid lipid domains in preference for adjacent fluid domains. A similar long-range ordering of protein distribution has been seen (2) at the onset of a spin label-predicted lateral phase separation in vesicles of 65:35 dielaidoyl phosphatidylcholine/dipalmitoyl phosphatidylethanolamine containing glycophorin. In this case the lipid distribution cannot be followed directly since the mixture is one which does not show line patterns in electron micrographs. The occurrence of this phenomenon is not restricted to model systems: Kleemann and McConnell have shown that intramembranous particle distribution in the inner membrane of a β-oxidation-deficient fatty acid auxotroph of *E. coli* is closely related to the spin label-predicted phase behavior of the bulk lipids (3). In these latter two cases the patches of intramembranous particles presumably mark the location of fluid lipid domains.

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