Lipid Modulation of Membrane Protein Function

Membrane Group Joint Anniversary Colloquium with the Canadian Biochemical Society, organized and edited by J. C. Ellory (University of Oxford)

Modelling the biological membrane

T. D. MADDEN, M. J. HOPE and P. R. CULLIS
Department of Biochemistry, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada

Generally, the complexity of biological membranes precludes the detailed analysis of individual components in situ. It has been common practice therefore to use model systems which mimic the native membrane but where one or two components can be studied in isolation. These vary from simple aqueous dispersions of lipid to integral proteins reconstituted in artificial vesicles. Many of these model systems, however, only poorly represent the native membrane, and important functional characteristics such as lipid and protein asymmetry and the presence of a membrane potential are often ignored. It has been our goal, therefore, to generate increasingly sophisticated models which mimic to a greater and greater extent the biological membrane, but in which the function and properties of each component are clearly understood. Below are outlined some characteristics of cell membranes and the strategies we have adopted to recreate them in model systems.

Large unilamellar vesicles

Upon hydration most phospholipids adopt a bilayer organization, and form large (> 1 μm) multilamellar vesicles (MLVs) consisting of concentric bilayers separated by narrow aqueous channels (Bangham et al., 1965). While such structures are useful membrane models for physical studies such as X-ray diffraction, differential scanning calorimetry or 31P-n.m.r., providing information on membrane permeability, thermotropic phase transitions (Ladbrook & Chapman, 1969) and lipid polymorphic phase preferences (Cullis & deKruijff, 1979), unilamellar vesicles are clearly a more representative model of cell membranes. Small unilamellar vesicles (SUVs) prepared by sonication of MLVs (Huang, 1969), while overcoming some of the limitations of MLVs, present difficulties due to their high radius of curvature, which results in lipid packing problems, and their low trapped volume. These limitations are circumvented by large unilamellar vesicles (LUVs) (0.1-1 μm diameter) which are perhaps the most realistic model for cell membranes. While a variety of techniques are available for producing LUVs, they often involve lengthy dialysis (Mimms et al., 1981) or require the use of organic solvents (Olson et al., 1979). Recently, however, we have developed a procedure whereby LUVs can be prepared directly from MLVs by extrusion through polycarbonate filters of defined pore size (Hope et al., 1985). By varying the filter pore size vesicles ranging in diameter from 50 to 400nm can be prepared (Mayer et al., 1986a). This procedure is rapid with preparation times typically less than 15 min and can be used with any lipid mixture that adopts a bilayer organization upon hydration. In subsequent sections the use of these LUVETS (large unilamellar vesicles by extrusion techniques) to form more sophisticated model membranes is described.

Membrane electrochemical gradients

A major function of biological membranes is to provide a permeability barrier allowing the creation of ion concentration gradients and consequently a membrane potential. Examples are the mitochondrial inner membrane, chromaffin granules, chloroplasts, sarcoplasmic reticulum and the plasma membrane. Such electrochemical gradients can also be created in model systems by establishing a concentration gradient of an entrapped permeant ion (e.g. K+ in the presence of valinomycin) between the vesicle interior and the external medium, thus creating a membrane potential (ΔΨ). Proton gradients (ΔpH) also give rise to membrane potential. The quantification of the membrane potential obtained is commonly achieved using probes (e.g. methyltriphosphonium or methylamine) which redistribute between the vesicle interior and exterior on the basis of the Nernst equation (for ΔΨ) or the Henderson-Hasselbach equation (for ΔpH). The accumulation of probe within the vesicle interior can be substantial. For example, a membrane potential of ~ 100 mV results in a 50-fold higher methyltriphosphonium concentration inside as compared with outside. As probe redistribution can occur within minutes (Bally et al., 1985) it is clear that the accumulation of lipophilic cations represents a rather effective membrane transport process. As a large number of drugs are lipophilic cations, including antineoplastic agents, local anaesthetics, β-blockers and biogenic amines such as dopamine, adrenaline and 5-hydroxytryptamine, this observation led us to propose that such molecules might also redistribute in response to an electrochemical gradient. In Fig. 1, the accumulation of adriamycin by LUVETS in response to a proton gradient is illustrated. Uptake is rapid even when cholesterol (50 mol %) is included in the vesicles and under appropriate conditions up to 98% of the available drug is sequestered (Mayer et al., 1986b). Similarly, rapid accumulation of vinblastine (Mayer et al., 1985a), dibucaine (Mayer et al., 1985b), dopamine and adrenaline (M. B. Bally, L. D. Mayer, H. Loughrey, T. Redelmeier, T. D. Madden, K. Wong, M. J. Hope & P. R. Cullis, unpublished work) have also been observed in response to a membrane potential. In the case of uptake driven by a K+ diffusion potential (membrane potential) an associated pH gradient is also present leading to the possibility that redistribution occurs via the neutral form of the lipophilic amine in accordance with the Henderson-Hasselbach equation (T. E. Redelmeir, M. B. Bally, L. D. Mayer, M. J. Hope, H. Loughrey & P. R. Cullis, unpublished work).

These observations have a number of important implications for cell membranes. First, local anaesthetics such as

Abbreviations used: MUVs, multilamellar vesicles; SUVs, small unilamellar vesicles; LUVs, large unilamellar vesicles; LUVETS, large unilamellar vesicles by extrusion techniques; TNBS, trinitrobenzene sulphonic acid.

619th MEETING, CAMBRIDGE  75