Membrane Processes

Molecular Biology and Medical Applications

Edited by
Gh. Benga H. Baum F.A. Kummerow

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With 84 Figures

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Preface

The burgeoning interest in biomembranes in recent years has been such that "membranology" is now virtually a subject in its own right, cutting vertically, as it were, through the strata of conventional disciplines from mathematics and physics, through chemistry, to biology. The very scope of the topic is thus so daunting that it is tempting to treat it only at one stratum of this hierarchy, be it the biophysics of phospholipid bilayers or the biochemistry of interactions at the cell surface.

Such an approach is entirely valid, particularly among specialists with common interests. However, this approach does present a distorted perspective to the newcomer to the field, and, more significantly, it fails to stimulate cross fertilization of ideas among workers at the various disciplinary levels. For example, as in all areas of molecular biology, the clinicians are frequently unaware of the contributions to their problems that might be made by the application of more basic knowledge and techniques. Conversely, biochemists or biophysicists may be ignorant of the existing practical problems to which they might address their expertise.

The present collection of chapters is intended as a contribution to such cross fertilization. It is by no means a comprehensive treatise on all aspects of membranology, rather it is a sampling of the status of selected topics at different levels, selected to illustrate the interconnections that become apparent between basic and applied biology when a common theme is recognized. The volume provides contributions for reference purposes at the professional level and aims broadly at biologists, biochemists, biophysicists, physicians, and so forth; that is, those who are active investigators working on cell membranes. We hope it will also be of great help to teachers and students at both the undergraduate and graduate levels.

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Part I

Structural and Functional Relationships in Cell Membranes

1
Reconstituted Membrane Systems

J.M. Wrigglesworth

This chapter is concerned with describing recent advances in the study of membrane systems by reconstitution methods. Particular emphasis is placed on the use of artificial phospholipid structures in the reconstitution of vectorial reactions catalyzed by membrane-associated enzymes.

A widely used approach to the study of enzyme systems is to fractionate and isolate the individual components of the system for further investigation. The resolved components can then be analyzed in isolation from other components to give specific information about individual catalytic reactions. A major disadvantage of this approach is that functional interactions among the different components are lost, especially in membrane-associated systems in which vectorial characteristics are important. In reconstitution studies, an attempt is made to reconstitute the original physiological process from the isolated components. In this way interactions among the different components and the importance of asymmetry and orientation in the membrane can be investigated. This approach is now being used on an increasing number of membrane-associated enzyme systems (Table 1.1), but probably its greatest success has been in investigations into the mechanism of coupling electron transfer reactions in mitochondria to the synthesis of adenosine triphosphate (ATP). In this chapter, we will mainly be concerned with examples from this area.

The success of the reconstitution approach for membrane systems has depended experimentally on the availability of suitable artificial membranes. With this in mind, the development of the liposome concept will first be reviewed together with the development of other, less widely used, artificial membrane systems. Before the full potential of membrane reconstitution could be realized, however, it is necessary to have a theoretical framework covering the concept of vectorial reactions. This was provided by the chemiosmotic theory of Mitchell (1961), and various consequences of this theory relevant to reconstitution studies will also be discussed. Finally, the question of how vectorial reactions in reconstituted systems relate to current ideas on the organization of membrane systems in vivo will be explored.

Table 1.1. Some Membrane Associated Enzyme Systems Investigated by Reconstitution Methods Using Model Membrane Structures

System	Membrane	References
H^+-ATPase	Liposome	Kagawa and Racker (1971)
	Planar bilayer	Drachev et al. (1974)
	Collagen membrane	Blanchy et al. (1979)
NAD-CoQ reductase	Liposome	Ragan and Hinkle (1975)
$CoQH_2$-cytochrome c reductase	Liposome	Leung and Hinkle (1975)
Cytochrome oxidase	Liposome	Hinkle et al. (1972); Jasaitis et al. (1972)
	Planar bilayer	Drachev et al. (1974)
Ca^{2+}-ATPase	Liposome	Racker and Eytan (1973)
Na^+/K^+-ATPase	Liposome	Goldin and Tong (1974)
Rhodopsin	Liposome	Davoust et al. (1979)
Bacteriorhodopsin	Liposome	Racker and Stoeckenius (1974)
	Planar bilayer	Kayushin and Skulachev (1974)
α-glutamyltranspeptidase	Liposome	Sikka and Kalra (1978)
Microsomal electron transfer components	Liposome	Strittmatter et al. (1978)
Chlorpolast pigments	Liposome	Mangel (1976)
	Planar bilayer	Ilani and Berns (1972)
Bacteriochlorophyll	Planar bilayer	Barsky et al. (1976)
Glucose transport	Liposome	Fairclough et al. (1979)
Band III anion transporter	Liposome	Rothstein et al. (1975)
Mitochondrial transhydrogenase	Liposome	Rydström et al. (1975)

Liposomes

Bangham (1963) was among the first to demonstrate that when phospholipids above a certain chain length are exposed to a large excess of aqueous medium they spontaneously arrange themselves into multilamellar concentric bilayer vesicles (Fig. 1.1) termed liposomes. Liposomes have been extensively studied as models for biological membranes (Bangham et al. 1965a, 1965b, 1965c; Bangham and Papahadjopoulos 1966; Bangham et al. 1967; Johnson and Bangham 1969a, 1969b), but more recently other approaches have been developed. Their potential to act as drug carriers for therapeutic use has been explored (Tyrrell et al. 1976; Colley and Ryman 1976; Finkelstein and Weissman 1978; Papahadjopoulos 1978). The possibility of introducing surface receptors into liposomes to target entrapped materials to specific cells in the body has been investigated (Gregoriadis and Neerunjun 1975; Juliano and Stamp 1976), and liposomal membranes have provided a framework for a multitude of reconstituted enzyme systems (Table 1.1).

Not all phospholipids are suitable for liposome formation. The ability of

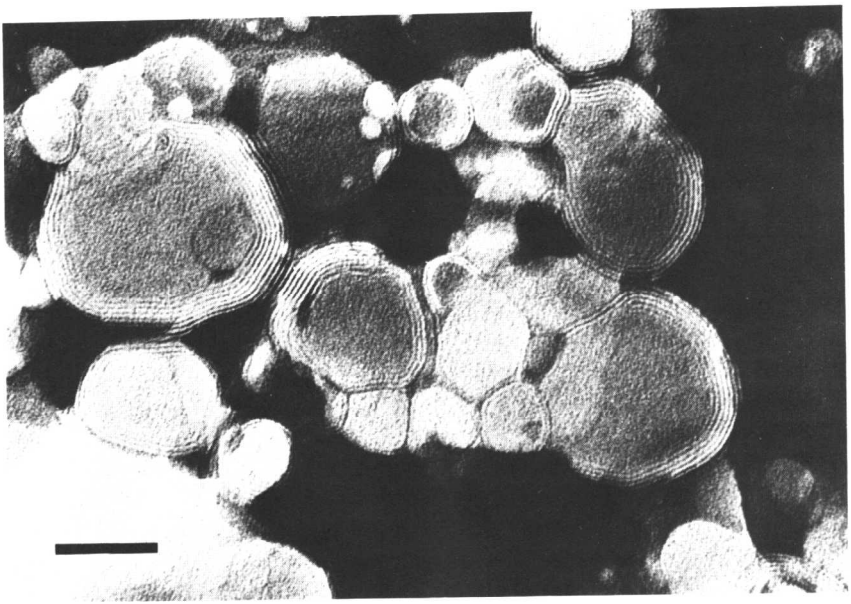

Figure 1.1. Multilamellar bilayer structures formed by mechanical agitation of phospholipids in salt solution. A mixture of phosphatidylcholine and phosphatidic acid (3:1) in a chloroform–ethanol solution was dried under nitrogen and hydrated by the addition of 50 m*M* potassium phosphate with mechanical agitation followed by brief (< 1 min) sonication. Two percent phosphotungstate, pH 7, was used for negative staining. Bar indicates 100 nm.

amphiphiles to form a "mesomorphic phase," or bilayer, is dependent on certain geometrical limitations (Israelachvili and Mitchell 1975; Israelachvili et al. 1976). Single-chained phospholipids generally form micellar structures, whereas double-chain phospholipids above a critical chain length form bilayers. This ability correlates with the very low critical micelle concentration of biological phospholipids compared to most single-chained amphiphiles. In addition, the hydrocarbon chains on the phospholipid have to exist in a liquid state (i.e., above their transition temperature) for successful bilayer formation (Chapman and Fluck 1966). Phospholipids bearing a net charge generally have to be "diluted" with neutral lipid. With these constraints satisfied, the ability to form lamellar structures appears general among the various phospholipids.

Mechanically shaken dispersions of suitable phospholipids form multilamellar liposomes, but prolonged sonication will form an aqueous dispersion of mainly unilamellar vesicles heterogeneous in size ranging in diameter from 250 to 1500 Å (Fig. 1.2). A preparation of uniform size can be isolated by molecular-sieve chromatography (Huang 1969) or by ultracentrifugation procedures (Barenholz et al. 1977). The smaller vesicles approach a lower limit of size where

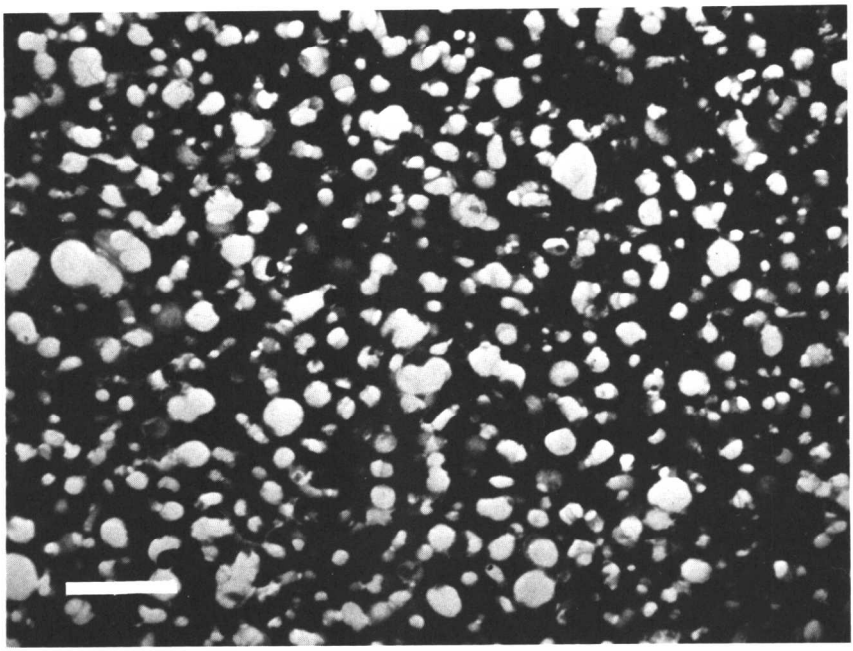

Figure 1.2. Unilamellar vesicles of phospholipid formed by prolonged sonication. Phosphatidylcholine and phosphatidic acid (2:1) were hydrated in a solution of 10 mM sodium chloride pH 6 and subjected to intermittent sonication for a total time period of 20 min in a probe sonicator on ice. Four percent ammonium molybdate, pH 7, was used for negative staining. Bar indicates 200 nm.

packing constraints prevent any further vesicle shrinkage (Cornell et al. 1980). Minimal size phosphatidylcholine vesicles have an average Stokes radius of 11 nm, with approximately 1100 phospholipid molecules in the inner layer of the bilayer and 1900 in the outer layer (Brouillette et al. 1982). The distribution of charged phospholipids between the two membrane surfaces is strongly dependent on the size of the vesicle. In small vesicles, the internal radius of curvature causes strong electrostatic repulsion between charged headgroups, and charged phospholipids appear to partition in the outer layer preferentially (Michaelson et al. 1973). However, this effect strongly depends on the mol% composition of the mixture (Barsukov et al. 1980) and would be expected to be strongly influenced by vesicle size and the presence of protein. It is interesting to note that for these small vesicles, the internal volume of 1.3×10^{-24} m^3 (or 1.3×10^{-21} L/vesicle) would allow for only one molecule of solute/vesicle at 1 mM solute concentration.

Methods other than sonication or mechanical dispersion have been used successfully to form phospholipid vesicles. Many of these rely on exposing an or-

ganic solution of the lipid to an excess of aqueous medium. Large (up to 1 μm in diameter) unilamellar vesicles have been produced by this method (Mueller and Rudin 1968; Deamer and Bangham 1976; Nichols et al. 1980). Dispersing the phospholipids in an aqueous medium with detergent followed by dilution (Racker et al. 1975) or removal of the detergent by column chromatography or dialysis (Kagawa et al. 1973) can result in a relatively uniform dispersion of vesicles. A suitable detergent has to be chosen. Cholate or the nonionic detergent octyl glucoside are often used since their relatively high critical micellar concentrations allow for easy removal by dialysis, but other detergents such as Triton X-100 (Wolosin 1980) and lysophosphatidylcholine (Eytan et al. 1975) have been used.

A critical factor in vesicle formation is the detergent-to-lipid concentration. This must be sufficiently high to disperse the phospholipid fully before dialysis. Mimms et al. (1981) found that dialysis of egg lecithin and octyl glucoside dispersions resulted in unilamellar vesicles when the detergent:lipid ratio was kept above 5:1. When lower ratios were used, much of the lipid was found in multilamellar aggregates. This is consistent with the results of Jackson et al. (1982) who followed the solubilization of large unilamellar egg phosphatidylcholine vesicles on the addition of octyl glucoside. Full conversion of bilayers into mixed micelles was only found above a detergent:lipid ratio of 3:1.

In the presence of hydrophobic proteins, many of the physical properties of liposomes are altered. To the author the most perplexing observation, often seen when membrane proteins are incorporated into phospholipid bilayers, is the influence of the protein in "directing" the phospholipid to form uni- rather than multilamellar vesicles. Kagawa et al. (1973) report that multilayered myelin figures are produced by the cholate-dialysis method when only phospholipids are present; when certain hydrophobic proteins are included in the detergent mixture, dialysis often produces smaller unilamellar vesicles. Similar findings have been reported by MacDonald and MacDonald (1975) in which the assembly of phospholipid vesicles incorporating sialoglycoprotein from the erythrocyte membrane was shown to result in unilamellar structures in contrast to multilamellar vesicles obtained with lipid alone. Liposomal systems incorporating different membrane proteins are found to respond differently to the various reconstitution procedures (Racker 1979), and the assembly of vesicular structures in the presence of hydrophobic protein is also reported to be strongly influenced by the composition of the phospholipid (Kagawa et al. 1973). Variations in detergent:lipid ratios may account for some of these effects (Mimms et al. 1981), but the conclusion from these and other studies must be that at present it is prudent to take an empirical approach for each multicomponent system under investigation.

Phospholipid vesicles, however prepared, are freely permeable to water and therefore subject to osmotic forces. Large liposomes will change volume in response to the concentration gradient of impermeable solutes (Bangham et al. 1967), but volume changes in preparations of the smaller size of vesicles are

restricted by packing density and surface tension considerations. The mechanical strength of the smallest of the membrane vesicles may enable them to support quite large differences in osmotic pressure (Walter 1975; but see Tanford 1979).

In view of the importance of ion transport concentration gradients in cellular bioenergetics, it is of interest to investigate whether artificial systems can serve as good models for such studies. Biological membranes appear to have high permeability coefficients for cations compared to most model systems (Deamer 1982), probably arising from different membrane mechanisms for ion translocation. Model systems have relatively low sodium and potassium ion permeability coefficients (10^{-13}-10^{-14} cm/s), but the passive permeability of artificial lipid bilayers, including liposomes, to protons is the subject of some disagreement. Values ranging from 10^{-4} cm/s (Nichols et al. 1980; Nichols and Deamer 1980; Clement and Gould 1981; Biegel and Gould 1981) to 10^{-9} cm/s (Nozaki and Tanford 1981; Gutknecht and Walter 1981) have been reported. However, Deamer (1982) points out that despite these conflicting data, the values are all still lower than the measured proton permeability of natural membranes. At a first approximation, it would seem that model systems do provide a sufficient barrier to proton flux for useful reconstitution experiments.

Black-Lipid Membranes and Other Model Membrane Systems

Black-lipid membranes can be formed by stroking a brush loaded with a solution of suitable lipids over a small hole in a hydrophobic barrier between two aqueous compartments (Bangham 1968). The term "black-lipid membrane" arises from the reflectance properties of the thin lipid film. The physical properties of these membranes have been extensively studied (Mueller et al. 1962; Mueller et al. 1964; Finkelstein and Cass 1968). They have a bimolecular leaflet structure similar to the liposome but usually also contain significant amounts of organic solvent, especially in the boundary region in which the film thickens as it attaches to the solid hydrophobic support (Henn and Thompson 1967; Pagano et al. 1972). The organic solvent can affect the electrical properties of the bilayer (Haydon et al. 1977) and also may affect the properties of any incorporated protein system. Black-lipid membranes are not nearly as stable as liposome bilayers; but despite the many difficulties associated with their use, they have been the chosen method for many successful studies on bilayer permeability. With this system it is possible to measure electrical potential differences and transport of material across a phospholipid bilayer separating two bulk aqueous phases.

Other hydrophobic material can easily be introduced into the bilayer. Mueller and colleagues (1962) were the first to study the ion permeability effects of added protein to black-lipid membranes. They showed that the addition of suitable proteins to the bilayer can induce voltage-dependent conductance proper-

ties similar to those found in biological membranes. These studies have been extended by Mueller and other workers (Mueller and Rudin 1968; Ting-Beall et al. 1979). Reconstitution studies of membrane-associated enzyme systems using black-lipid membranes have allowed direct measurements to be made of electric current generation by cytochrome oxidase, adenosine triphosphatase (ATPase), and bacteriorhodopsin (Drachev et al. 1974; Dancshazy and Karvaly 1976; Issaurat et al. 1980).

Other phospholipid systems have proved less useful in reconstitution studies. The limitations of using monolayers for investigating vectorial reactions is obvious, but they can be used to provide details of specific protein-lipid and lipid-lipid interactions that cannot be measured in less well-defined structures (Redwood and Patel 1974; Wooster and Wrigglesworth 1976; Quinn and Esfahani 1980). The use of lipid-impregnated filters as an artificial framework for reconstitution studies has been explored with some success by Skulachev and co-workers (Skulachev 1976; Konstantinov et al. 1980). These filters provide the advantages of the black-lipid membrane system without the instability problems inherent in unsupported bilayers.

Electrochemical Gradients and Ionophores

The importance of electrochemical gradients in cell physiology has long been known from studies on the cation balance in cells, especially the nerve cell. However, a shift in paradigm was necessary before the general role of vectorial reactions in metabolism could be realized. This was provided by Mitchell in his chemiosmotic theory (Mitchell 1961). The theory sets out to explain how certain chemical reactions can be linked by a suitable vectorial arrangement of the reactants and products (Fig. 1.3). According to the theory, the coupling between redox reactions and ATP synthesis occurs via the intermediate of an electro-

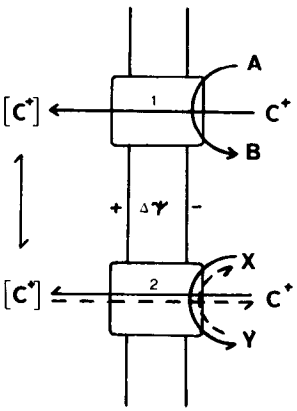

Figure 1.3. Coupling of scalar chemical reactions by a vectorial flow of reactants across a membrane. Both reactions 1 and 2 can translocate compound C across the membrane. If either or both of the reactions are reversible, then the gradient of C formed by one reaction can be used to reverse the other. When C is charged, the electrochemical gradient of C will depend on the concentration difference of C across the membrane and the membrane potential ($\Delta\psi$).