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edited by

D. CHAPMAN

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Preface

There are scientists who believe that there is now little more to be determined concerning biomembrane structure and function. "A bilayer of lipid containing intrinsic proteins with some extrinsic proteins attached—add a little mobility here and there and the story is complete". In my opinion, and those of us concerned with the study of biomembranes, this is not the case. Some of the most fundamental properties characterising biomembranes are still not understood. Lipid class asymmetry, ion pumping mechanisms, the role of the highly unsaturated lipids in nerve excitable membranes—these are a few obvious areas of uncertainty which in our opinion require clear cut answers. The authors of this volume point to many other unresolved questions.

This series on Biological Membranes is intended to help to summarise, at regular intervals, the existing situation and to point to these unanswered questions. It is hoped that the volumes will be useful to many scientists in biochemistry, biophysics, physiology and medicine. A knowledge of the fundamentals of biomembranes may in turn enrich our understanding of those disease conditions which appear to be linked with abnormal biomembrane function.

December 1981

D. CHAPMAN

Introduction

The last few years have seen considerable progress in our knowledge of biomembrane structure and with it improvements in our understanding of the many functions associated with biomembranes and cell surfaces. There is now broad agreement about many of the general aspects of biomembrane structure—the role of the lipid matrix, the presence of extrinsic and intrinsic protein and the cytoskeleton which occurs with some cell membranes. Since the first volume of this series, various concepts then put forward have become increasingly refined as improved physical and biochemical methods have been applied, and thinking has become clearer and better defined. For example, the concept of biomembrane fluidity put forward in 1966 by Chapman and co-workers is now expressed in terms of static and dynamic disorder. The suggestions by Singer and Wallach of intrinsic protein structure have been shown to be correct and in some cases detailed structures proposed in terms of amino acid sequences for these proteins.

Yet there are questions and problems remaining to be resolved underneath the broad generalisation that we presently enjoy. Some of these questions are discussed by the authors of the present volume of Biological Membranes.

What is the role of the glycolipids in biomembrane structure and function? How are they involved in biomembrane asymmetry in receptor processes in tissue development? What is the importance of the ceramide portion of these lipids? These are some of the questions discussed by K.-A. Karlsson in Chapter 1.

The structure and the synthesis of integral (or intrinsic) proteins is presently a very active topic. The monotopic proteins such as cytochrome b_5, the bitopic proteins such as the erythrocyte sialoglycoprotein and the polytopic proteins such as bacteriorhodopsin are all discussed by C. J. Brock and M. J. A. Tanner in Chapter 2.

Theoretical models of biomembrane structure are often presented nowadays by theoretical physicists who attempt to present a firm mathematical basis for the physics of the lipid matrix to predict transition temperatures, disorder, the consequence of random and non-random arrangements of the various components and the perturbations introduced by cholesterol and proteins. In Chapter 3, D. A. Pink describes the theoretical methods and their applications.

When we look closer at the fine details of intrinsic protein–lipid interactions, we see that there have been confusing views put forward. A concept of stoicheiometric protein–lipid complexes has existed and dominated much biochemical thinking. In particular, the concept of the annulus lipid, the long-lived shell of lipid attached to and surrounding the hydrophobic core of the intrinsic protein, has been readily accepted by many biochemists. Its role in eliminating cholesterol, controlling enzyme activity, even when excess lipid is present, have all been actively and persuasively expressed. Recent nuclear magnetic resonance evidence and the application of other physical techniques to the study of this problem are discussed in Chapter 4 by D. Chapman.

When we turn to the relationship of biomembrane structure and function, we find many important studies being carried out. In Chapter 5 studies of the lymphocyte plasma membrane are described. The intrinsic and extrinsic proteins of this membrane and the importance of the cytoskeleton are examined. The inhibition of lymphocyte growth and the effect of calcium ions are considered by A. P. Johnstone and M. J. Crumpton.

The immune system and the various interactions between complement proteins and biomembranes are being actively studied. Important clarification of the processes involved are being derived. A. E. Esser in Chapter 6 defines the complement pathway, the activation of complement on biomembranes and the various models by which lysis is thought to occur.

Hormones and plasma membrane receptors are discussed in Chapter 7 by B. A. Cooke. Cholera toxin as a hormone model and the involvement of cyclic AMP in hormone action are considered. The hormone–receptor–adenylate cyclase system is examined in detail and the problems associated with the present models discussed.

Biomembrane fusion is nowadays receiving renewed interest arising from the fundamental principles involved in the fusion process and also in the application of fusion processes to problems associated with hormone action, monoclonal antibodies and possible technological applications.

How are non-lamellar structures involved in biomembrane fusion? Are the present techniques of spectroscopy suitable for showing the principles of the fusion processes. What are the appropriate chemical structures which bring about biomembrane fusion? These questions are discussed by J. A. Lucy in Chapter 8.

The problem of the principles involved in drug action and biomembranes is clearly an important one and must receive increasing study. Yet drug molecules have so many disparate structures and the generalisations are not always obvious. The various theories which have been proposed for general anaesthetic action particularly with regard to lipid theories are examined by A. S. Janoff and K. W. Miller in Chapter 9.

The study of the cell surface, the dynamics of biomembranes, the trigger processes, and the various functions associated with them are important not only for their intellectual and scholastic value, but also for a better understanding of health and disease conditions as well as for future technologies. I hope that the chapters in this volume by the present contributors will add further stimulation and value to this important field.

Contents

Chapter 1

Glycosphingolipids and Surface Membranes

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I. Introduction

Present evidence is in favour of a localisation of sphingolipids to the outer half of the surface membrane bilayer. The characteristic structural features of the lipophilic part, ceramide, and the complexity and variability of the carbohydrate part of glycolipids are probably essential for the functional integrity of animal cells in relation to their environment. The actual functions may include controlled permeability, reception of signals and specific cell to cell contacts.

Although several interesting biological phenomena have been found associated with glycolipids at the cell surface and these results have stimulated a large number of investigations, this field of research is certainly only at its beginning. The medically important finding of the specific binding of cholera toxin to a particular glycolipid demonstrates that an external ligand may produce an effect in the cell through binding to a receptor that does not span the membrane.

At the present stage of development, important suggestions on the possible functions of membrane components may primarily develop from comparative biochemical studies. The knowledge of a variation in structure and concentration of a substance in relation to tissue function and compartmentalisation may be an essential prerequisite for the formulation of adequate working models to test more precise functions. In this way, one should carefully consider both the non-polar part, anchored in the membrane, and the carbohydrate part, the non-reducing end of which is probably positioned at a variable distance from the membrane matrix.

The present chapter is not intended to be a review in the traditional sense, with a reference list completely covering the appropriate literature. Rather, I shall consider old and recent data, some of them not enough appreciated before, that demonstrate the unique position of glycosphingolipids among membrane lipid components and put them together to make reasonable working models. Reviews have appeared on separate aspects of sphingolipids. There are rather old reviews on sphingolipid fatty acids (Mårtensson, 1969) and long-chain bases (Karlsson, 1970a,b), and more recent ones on glycolipid saccharides (Haines, 1971; Hakomori and Kobata, 1974; Sweeley and Siddiqui, 1977; Ledeen, 1978; McKibbin, 1978) and glycolipids in tumour tissue (Bergelson, 1972; Brady and Fishman, 1974; Hakomori, 1973, 1975a,b; Wallach, 1975; Critchley and Vicker, 1977).

II. Existing Types of Sphingolipids

Different sphingolipids defined in their different variants of linkage to the long-chain base are summarised in Fig. 1. It is not known whether compounds with a free amino group (Fig. 1A) or free ceramide (Fig. 1B) have a role in the final membrane or are metabolic intermediates. Free base has been found in yeast (Karlsson, 1970a; Kimura *et al.*, 1974) and malignant melanoma (K.-A. Karlsson and B. E. Samuelsson, unpublished results). Glucosylsphingosine was identified in spleen of a human case with Gaucher's disease (Oshima, 1976), globotriaosylsphingosine and globotetraosylsphingosine are present in human erythrocyte (K.-A. Karlsson and G. Larson, unpublished results), and fucose-containing oligoglycosylsphingosine was identified in cultured cells (Skelly *et al.*, 1976). Free ceramide (Fig. 1B) exists in several

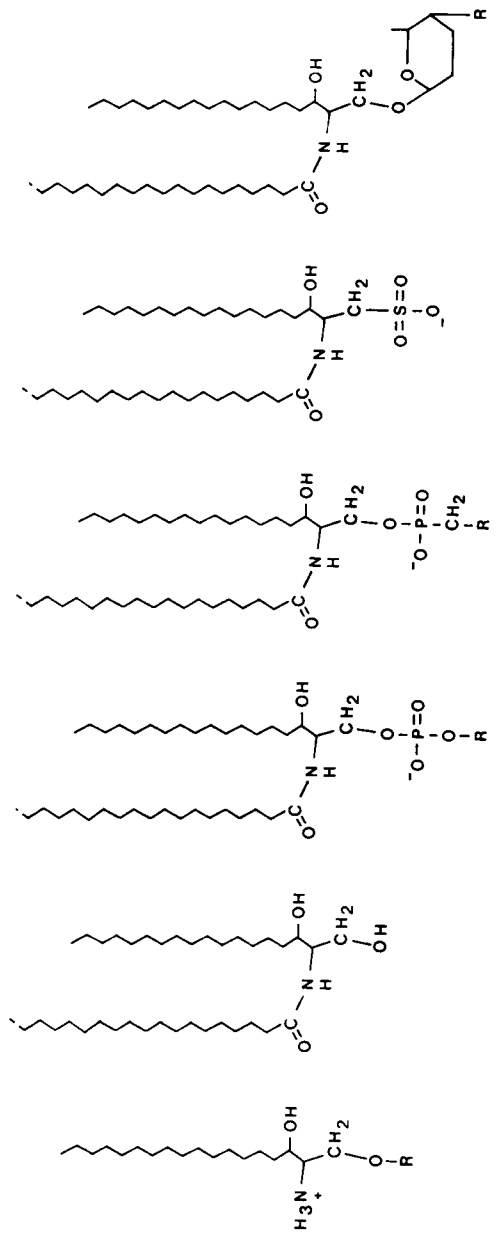

FIG. 1. Different types of sphingolipids as defined by their various bonds to the long-chain base.

mammalian organs (see Bouhours and Guignard, 1979), in plants (Fujino and Ohnishi, 1976), and bacteria (Miyagawa *et al.*, 1979). Ceramide is absent from human erythrocyte (only a plasma membrane) but is one of the major sphingolipids of human malignant melanoma, composed of rapidly growing cells (K.-A. Karlsson and B. E. Samuelsson, unpublished results).

The vast majority of sphingolipids have the long-chain base substituted at both the amino group and the hydroxy group at carbon atom one (Fig. 1C–F). In sphingomyelin and some other phosphosphingolipids (Ansell *et al.*, 1973), there is a phosphodiester group (Fig. 1C). This linkage is also present in complex glycosphingolipids in plants (Hitchcock and Nichols, 1971; Ansell *et al.*, 1973; Laine *et al.*, 1980) and probably in protozoa (Wilhelms *et al.*, 1974; Dearborn *et al.*, 1976). Several kinds of phosphonates (Fig. 1D) have been detected (Ansell *et al.*, 1973; Karlsson and Samuelsson, 1974; Matsuura, 1979) and a unique ceramide sulphonate (Anderson *et al.*, 1975), Fig. 1E.

In known molecular species of sphingolipids, the dominant part is made up of glycolipids with a glycosidic bond to sphingosine (Fig. 1F). The structure and function of these is the major subject of this chapter.

III. Glycosphingolipid Classes

A great number of different monosaccharides has been identified in glycosphingolipids of various origins (Table I). As some glycolipids have been suggested to carry up to 60 sugar residues (see Kościelak *et al.*, 1976a), this means that the monosaccharides listed may be theoretically combined in a tremendous number of glycolipid species. A simple calculation for disaccharides of two different hexoses gives about 70 isomers, as the glycosidic bond varies in both position and configuration and the heterocycle may be five- or six-membered. This may be compared with peptides where only two possibilities exist for two different amino acids. A great variability may also mean a rich biochemical language and this is one reason why surface-membrane carbohydrates are being considered in biological recognition (see below).

Although the known structures of natural glycolipids rapidly grow in number, we do not expect all indicated possibilities to exist. The glycosyltransferases working during biosynthesis (Morré, 1977; Dawson, 1978) have been shown to have an extremely high specificity in the addition of each monosaccharide to a growing saccharide chain (see also below). A particular cell may therefore strictly select its carbohydrate pattern on the surface. This may explain why certain classes or series of glycosphingolipids are found, often with a characteristic variation with tissue.

TABLE I

Monosaccharides identified as components of glycosphingolipids

Monosaccharide*	Origin	Reference†
Glc	Animal, plant, yeast	Hitchcock and Nichols (1971)
Gal	Animal, plant	Hitchcock and Nichols (1971)
GlcNAc	Animal, plant	Laine *et al.* (1980)
GalNAc	Animal	
Fuc	Animal, plant	Hitchcock and Nichols (1971)
NeuAc	Animal	
NeuGc	Animal	
Man	Animal, plant	Hori *et al.* (1977a,b); Laine *et al.* (1980)
Xyl	Animal	Karlsson *et al.* (1972); Hori *et al.* (1979)
Ara	Animal	Vaskovsky *et al.* (1970); Hori *et al.* (1979); Sugita (1979)
Rha	Animal	Vaskovsky *et al.* (1970)
GlcN	Plant	Hitchcock and Nichols (1971)
GlcU	Plants, bacteria	Hitchcock and Nichols (1971); Yamamoto *et al.* (1978)
3-0-Me-Gal	Animal	Araki *et al.* (1980)
4-0-Me-Gal	Animal	Hori *et al.* (1979)
3-0-Me-GalNAc	Animal	Matsubara and Hayashi (1978)
3-0-Me-Fuc	Animal	Hori *et al.* (1979)
8-0-Me-NeuGc	Animal	Sugita (1979)
4-0-Me-GlcU	Animal	Hori *et al.* (1979)
0-Ac-NeuAc	Animal	Haverkamp *et al.* (1977)
0-Ac-NeuGc	Animal	Hakomori and Saito (1969)

* The abbreviations follow a recent recommendation of nomenclature (IUPAC-IUB Commission, 1977).

† Only references for less common sugars or origins are given. For a review on mammalian tissues, see Sweeley and Siddiqui (1977).

Table II shows the monosaccharides found directly linked to ceramide. Of these only Glc and Gal are common. Table III illustrates the core structure of more common glycolipids selected from mammalian systems and Table IV gives examples of substitutions of these core glycolipids. An excellent review on the structure of mammalian glycolipids has recently appeared (Sweeley and Siddiqui, 1977). Although a systematic chemical study on glycolipids of different mammalian tissues is still lacking, the complexity as illustrated by these Tables is already large enough to suggest a significance of these carbohydrate specificities for recognition processes on the cell surface.

To make the reader aware of a rich variation in glycolipids also in lower animals I have added Table V. This shows examples of disaccharides found as parts of invertebrate glycolipids and not yet discovered in mammalian tissues. Some of these glycolipids may also contain phosphate, phosphonate

or sulphate. Certainly, the known number of glycolipid species is growing as a consequence of the development of preparative and analytical methods (see Section VIII, p. 58).

Additional examples of structures will appear in the following text on functional aspects.

TABLE II

Monosaccharides found in linkage to ceramide

Glycolipid	Reference
Glcβ1 → Cer	Sweeley and Siddiqui (1977)
Galβ1 → Cer	Sweeley and Siddiqui (1977)
Xyl1 → Cer	Karlsson *et al.* (1972)
Fucα1 → Cer	Watanabe *et al.* (1976)
GlcU1 → Cer	Yamamoto *et al.* (1978)

IV. Membrane Asymmetry and Sphingolipid Localisation and Concentration

A fascinating development in the field of membrane biochemistry is the establishment of an asymmetrical distribution of components between the two membrane layers (Bretscher, 1971; Rothman and Lenard, 1977; Bergelson and Barsukov, 1977; Op den Kamp, 1979), the functional significance of which is largely unknown. Regarding sphingolipids, these may be exclusively located in the outer monolayer. This has been demonstrated in erythrocyte for sphingomyelin (Renooij *et al.*, 1976; Op den Kamp, 1979), the major mammalian phosphosphingolipid, and for globoside (Gahmberg and Hakomori, 1973; Steck and Dawson, 1974), one of the major glycolipids of human tissues. With a sensitive histochemical method for the localisation of the glycolipid receptor for cholera toxin, an exclusive outside position was found for several cells, including epithelial cells of human small intestine and different nerve cells (Hansson *et al.*, 1977). Membrane-bound carbohydrate in general (glycolipid and glycoprotein) was shown to be surface located in human erythrocytes (Gahmberg, 1976). It is also a well known fact that blood group antigens, some of which are glycolipids (see below), are present on the cell surface of a number of cells. One may therefore assume that all surface membrane sphingolipids are exclusively located in the outer monolayer.

Concerning subcellular localisation, sphingolipids are generally considered to be plasma-membrane components (however, see below for tumours, p. 36). When found in intracellular membranes they may be associated with the formation of new plasma membrane (Rothman and Lenard, 1977).

TABLE III

Examples of core structures of mammalian glycolipids*

Glycolipid†	Proposed semisystematic name†	Trivial name
Simple members		
1. Galβ1 → 1Cer	Galactosylceramide	Cerebroside
2. Glcβ1 → 1Cer	Glucosylceramide	Cerebroside
3. Galβ1 → 4Glcβ1 → 1Cer *or* Lacβ1 → 1Cer	Lactosylceramide	Cytolipin H
Gala series		
4. Galα1 → 4Galβ1 → 1Cer	Galabiosylceramide	Digalactosylceramide
5. GalNAcα1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 1Cer		Forssman glycolipid
Globo series		
6. Galα1 → 4Lacβ1 → 1Cer	Globotriaosylceramide	P^k antigen, Fabry trihexosylceramide
7. GalNAcβ1 → 3Galα1 → 4Lacβ1 → 1Cer	Globotetraosylceramide	P antigen, Globoside, Cytolipin K
8. GalNAcα1 → 3GalNAcβ1 → 3Galα1 → 4Lacβ1 → 1Cer	Globopentaosylceramide	Forssman glycolipid, Forssman antigen
Isoglobo series		
9. Galα1 → 3Lacβ1 → 1Cer	Isoglobotriaosylceramide	—
10. GalNAcβ1 → 3Galα1 → 3Lacβ1 → 1Cer	Isoglobotetraosylceramide	Cytolipin R
Ganglio series		
11. GalNAcβ1 → 4Lacβ1 → 1Cer	Gangliotriaosylceramide	Asialo-GM2
12. Galβ1 → 3GalNAcβ1 → 4Lacβ1 → 1Cer	Gangliotetraosylceramide	Asialo-GM1
13. GalNAcβ1 → 4Galβ1 → 3GalNAcβ1 → 4Lacβ1 → 1Cer		
Isoganglio series		
14. Galβ1 → 3GalNAcβ1 → 3Lacβ1 → 1Cer‡		
Lacto series		
15. GlcNAcβ1 → 3Lacβ1 → 1Cer	Lactotriaosylceramide	
16. Galβ1 → 3GlcNAcβ1 → 3Lacβ1 → 1Cer	Lactotetraosylceramide	Lewis precursor