CRC

PHOSPHOLIPIDS *and* CELLULAR REGULATION

Volume I

J. F. Kuo

CRC PRESS

Phospholipids and Cellular Regulation

Volume I

Editor

J. F. Kuo
Department of Pharmacology
Emory University School of Medicine
Atlanta, Georgia

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data
Main entry under title:

Phospholipids and cellular regulation.

Bibliography: p.
Includes index.
1. Cellular control mechanisms. 2. Phospholipids.
3. Cell membranes. I. Kuo, J. F. (Jyh-Fa), 1933-
QH604.P46 1985 574.87'5 84-28569
ISBN 0-8493-5537-0 (v. 1)
ISBN 0-8493-5538-9 (v. 2)

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

Second Printing, 1986

International Standard Book Number 0-8493-5537-0 (Volume I)
International Standard Book Number 0-8493-5538-9 (Volume II)

Library of Congress Card Number 84-28569
Printed in the United States

PREFACE

The importance of phospholipids in biological systems has become increasingly recognized and appreciated in recent years. These two volumes are intended to give a comprehensive and critical coverage of the progress made in certain major areas of research directly or indirectly related to phospholipids. Volume I begins with chapters dealing with the structural and functional roles of phospholipids in biological membranes and techniques for studying phospholipid membranes. More dynamic aspects of phospholipids follow, which include their roles in the modifications of the functional properties of membranes, in intracellular mediator systems, and in human disease. Volume II covers two biologically important phospholipids (i.e., platelet activating factor and antitumor agent alkyllysophospholipid), some major aspects of the newly discovered (thanks to Professor Nishizuka and his able colleagues in Japan) phospholipid/Ca^{2+}-dependent protein kinase (protein kinase C) system, including its role in transduction of membrane signals, and finally, regulation of oxidative enzymes by phospholipids and membrane. My deep gratitude is extended to many leading investigators in the fields, who have kindly contributed their chapters. I hope that the volumes will serve as a comprehensive and valuable source of references, thus fostering a further advancement in this ever rapidly expanding research on the roles of phospholipids in cellular function and regulation.

J. F. Kuo

THE EDITOR

J. F. Kuo holds the Ph.D. in Biochemistry from the University of Illinois (Urbana) and is Professor of Pharmacology at Emory University School of Medicine. His previous positions include Research Biochemist at Lederle Laboratories and Assistant and Associate Professor of Pharmacology at Yale University School of Medicine. He was a Visiting Professor of the Swedish Medical Research Council at Linköping University, Sweden, where he was awarded the D. Med. (hon.) in 1980. Dr. Kuo's research covers the biochemical, pharmacological, immunological, and pathophysiological aspects of membrane receptors, cyclic nucleotides, calcium, phospholipids, and protein phosphorylation systems. He has published extensively in these areas of investigation.

CONTRIBUTORS

Fulton T. Crews
Assistant Professor
Department of Pharmacology
University of Florida College of Medicine
J. Hills Miller Health Center
Gainesville, Florida

Pieter R. Cullis
Associate Professor
Department of Biochemistry
University of British Columbia
Vancouver, British Columbia
Canada

John N. Fain
Professor and Chairman
Section of Biochemistry
Brown University
Providence, Rhode Island

Robert V. Farese
Professor of Medicine
Director, Division of Endocrinology
and Metabolism
Department of Internal Medicine
University of South Florida
College of Medicine
and
Associate Chief of Staff
Research and Development
James A. Haley Veterans Hospital
Tampa, Florida

R. Adron Harris
Associate Professor
Department of Pharmacology
University of Colorado
Denver, Colorado

Robert J. Hitzemann
Associate Professor
Departments of Pharmacology and
Psychiatry
University of Cincinnati
Cincinnati, Ohio

M. J. Hope
Research Associate
Department of Biochemistry
University of British Columbia
Vancouver, British Columbia
Canada

Karl Y. Hostetler
Associate Professor of Medicine
Department of Medicine
University of California at San Diego
San Diego, California

B. de Kruijff
Professor
Institute of Molecular Biology
State University of Utrecht
Padualaan, Utrecht
The Netherlands

Irene Litosch
Research Associate
Division of Biology and Medicine
Section of Biochemistry
Brown University
Providence, Rhode Island

Horace H. Loh
Professor
Departments of Pharmacology and
Psychiatry
University of California
San Francisco, California

Alfred H. Merrill
Assistant Professor
Department of Biochemistry
Emory University School of Medicine
Atlanta, Georgia

J. Wylie Nichols
Assistant Professor
Departments of Biochemistry and
Physiology
Emory University School of Medicine
Atlanta, Georgia

C. P. S. Tilcock
Research Associate
Department of Biochemistry
University of British Columbia
Vancouver, British Columbia
Canada

A. J. Verkleij
Associate Professor
Institute of Molecular Biology
State University of Utrecht
Padualaan, Utrecht
The Netherlands

TABLE OF CONTENTS

Chapter 1

STRUCTURAL PROPERTIES AND FUNCTIONAL ROLES OF PHOSPHOLIPIDS IN BIOLOGICAL MEMBRANES

P. R. Cullis, M. J. Hope, B. de Kruijff, A. J. Verkleij, and C. P. S. Tilcock

TABLE OF CONTENTS

I. INTRODUCTION

A. Scope

Membranes contain an astonishing variety of lipids. The development of this diversity must result in significant evolutionary advantages to the uni- or multicellular organism in which they reside. This suggests particular functional roles for each component; however, clarification of these functional roles for individual lipid species has proven difficult. In this work we present an overview of the physical properties of phospholipids as demonstrated in model membrane systems and their possible functional roles in membrane-mediated phenomena.

The literature in this area is large and is growing rapidly. In order to achieve a basic coherence and form, we have largely restricted the scope of this review to the lipids of eukaryotic membrane systems. This has two major consequences. First, there is now a large body of evidence to suggest that most, if not all, eukaryotic membrane phospholipids are in the fluid liquid crystalline state at physiological temperatures. As a result, this work is largely restricted to the properties of liquid crystalline lipid systems. The gel-liquid crystalline characteristics of membrane lipids, which have been extensively reviewed elsewhere,[1] are only mentioned where germane. Second, where possible, we have concentrated on studies revealing the properties of lipids actually found in membranes. As a result, the literature on synthetic lipid systems is only included to the extent that it contributes to an understanding of the behavior of naturally occurring lipid species.

B. Roles of Lipids in Membranes: An Overview

Development of an understanding of the functional roles of lipids in membranes began with the early experiments of Gorter and Grendel,[2] who came to the conclusion that the erythrocyte contained sufficient lipid to provide a bilayer lipid matrix surrounding the red cell. The resulting concept that the major functional role of lipids is to provide a bilayer permeability barrier between external and internal compartments has remained a dominant theme in our understanding of biological membranes. Subsequent observations that membranes are fluid, allowing rapid lateral diffusion in the plane of the membrane,[3] and that membrane proteins are often inserted into and through the lipid bilayer have further contributed to our present understanding of membranes, resulting in the Singer and Nicholson[4] "fluid mosaic model". A refined version of this model is shown in Figure 1, which emphasizes the observations that membranes exhibit asymmetric transbilayer distributions of lipid and protein components. In the red cell membrane, for example, phosphatidylserine and phosphatidylethanolamine are largely localized to the interior monolayer,[5] whereas phosphatidyl choline, sphingomyelin, and the carbohydrate-containing moieties of glycolipids and glycoproteins are found on the membrane exterior. For completeness, the membrane potential, $\Delta\psi$, arising from transbilayer chemical gradients of ions such as Na^+, K^+, and H^+ is also indicated.

Within the terms of this model, the functional roles of lipids fall into categories related to an ability to self-assemble into bilayer structures on hydration, thus providing a permeability barrier as well as a matrix with which functional membrane proteins can be associated. Roles of individual lipid components may therefore concern establishing the bilayer structure itself, establishing appropriate permeability characteristics, satisfying insertion and packing requirements in the region of integral proteins, as well as allowing the surface association of peripheral protein via electrostatic interactions. All of these demands are clearly critical. An intact permeability barrier to small ions such as Na^+, K^+, and H^+, for example, is vital to establishing the electrochemical gradients. These give rise to a membrane potential which drives other membrane-mediated transport processes, whereas the lipid in the region of membrane protein must seal the protein into the bilayer so as to both prevent nonspecific leakage and to provide an environment appropriate to a functional protein conformation.

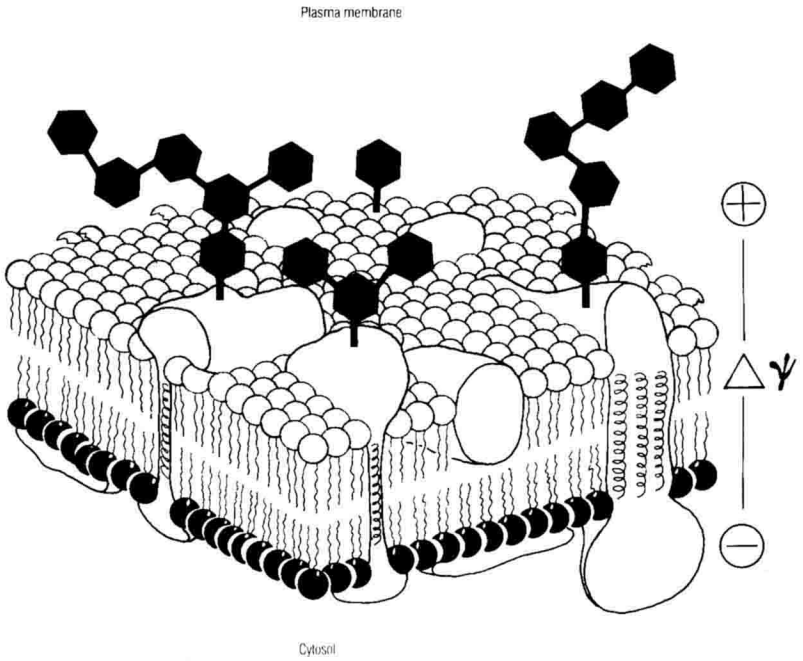

FIGURE 1. A refined fluid mosaic model of biological membranes. The transbilayer asymmetry of lipids and carbohydrates is emphasized as is the membrane potential $\Delta\psi$ arising from transbilayer electrochemical gradients of various ions.

C. Organization

The organization of this chapter stems from a model building approach to the understanding of the functional roles of individual lipid components of membranes. Briefly, the physical properties and roles of individual lipids are exceedingly difficult to ascertain in an intact biological membrane due to the complex lipid composition. In order to gain insight into the roles of individual components, much simpler "model membrane" systems are required which contain the lipid species of interest. The simplest of these model systems consists of the isolated lipid dispersed in an aqueous buffer, which can be used to determine structural and motional properties. At the next level of sophistication, unilamellar model systems are required to examine properties such as permeability. Subsequent, more sophisticated unilamellar models will then include reconstituted proteins if an understanding of lipid-protein interactions or the influence of a given lipid on protein function is required. This step-by-step development of increasingly sophisticated well-defined systems which model aspects of the composition and function of biological membranes should allow the properties and roles of individual components to be elucidated in an unambiguous manner.

This model approach dictates the development we follow here. First, some overview of methods of generating model membrane systems is required, as presented in the next section. Subsequently, the structural properties of lipids as elucidated in lipid-water dispersions are discussed. The fact that membrane lipids can adopt a variety of structures in addition to the bilayer organization leads to the possibility that the roles of certain lipids may be related to an ability to form nonbilayer structures. Membrane fusion is a particularly important example as indicated in Section V. The permeability properties of lipids and the roles of lipids in protein function as indicated by studies of reconstituted lipid-protein systems are reviewed in the following two sections. Finally, these observations are related to observations on intact biological membranes themselves in Section VIII.

II. MODEL MEMBRANE SYSTEMS

A. Dispersions of Lipids in Water

As indicated above, the simplest model system is obtained by depositing the lipid of interest as a film (normally by evaporation from chloroform), which is subsequently hydrated by mechanical agitation (e.g., vortexing), in the presence of aqueous buffer. In the case of lipids which adopt bilayer structure on hydration, the result is a milky suspension containing large "multilamellar vesicles" (MLVs) or liposomes.[6] These systems commonly range in size from 0.5 to 10 μm and consist of concentric lipid bilayers in an onionskin configuration. As little as 10% of the total lipid is contained in the outermost bilayer.

Lipids that adopt the hexagonal H_{II} phase (see Section IV) are usually much more difficult to disperse in water and form a fine particulate suspension which is easy to distinguish from MLV systems. The particles basically consist of a hydrocarbon matrix penetrated by aqueous channels (~20 Å diameter) which is surrounded by a monolayer of lipid with polar head groups oriented to the external aqueous medium.

B. Small Unilamellar Vesicles

The simple lipid-water dispersions are most useful for studies on the structural preferences of lipids and factors which modulate these preferences; however, they are clearly not accurate models of biological membranes in that MLVs contain internal bilayers, whereas H_{II} dispersions do not exhibit a well-defined permeability barrier between external and internal environments. Unilamellar systems provide more representative model systems. The simplest techniques for producing such systems generate "small unilamellar vesicles" (SUVs) which exhibit diameters in the range of 25 to 40 nm. Note that only lipid or lipid mixtures which form bilayer structure on hydration (e.g., MLVs on mechanical agitation) can form stable SUVs. The major techniques for making SUVs involve sonication[7] or passage through a French press. The small size of the vesicles produced has certain advantages. For example, high resolution nuclear magnetic resonance (NMR) spectra can be obtained from such systems, allowing outside-inside lipid distributions to be determined,[9] as well as details of acyl chain mobility;[10] however, these advantages are outweighed by the disadvantages concerning the very small radius of curvature which can perturb the physical properties of lipid components.[10] Related limitations concern the small interior aqueous volume, which limits the amount of material that can be trapped inside. For example, a vesicle of 25 nm diameter can contain only $\sim 1.6 \times 10^3$ atoms of a solute trapped at a nominal 1 m*M* concentration, assuming a bilayer thickness of 4 nm. Parameters such as internal pH are difficult to define, as illustrated by the observation that at pH = 7 only 1 in approximately 10 of these vesicles contained a free proton.

C. Large Unilamellar Vesicles

The difficulties encountered with the SUV systems have led to a substantial effort to obtain large unilamellar vesicles, which may be generally defined as unilamellar systems with diameters greater than 50 nm. Most of these procedures have been well reviewed elsewhere.[11] Briefly, the LUV techniques can be divided into three general classes consisting of vesicles prepared by detergent dialysis,[12] vesicles prepared employing organic solvents,[13-15] and LUVs prepared directly from MLV dispersions.[7,16] The LUVs thus produced range in size from 50 to 200 nm and each technique has various advantages and limitations. The detergent dialysis techniques involve solubilizing the lipid in the detergent of choice which is followed by dialysis to remove the detergent. This leaves unilamellar vesicles. Difficulties encountered concern lipid species which are difficult to solubilize, limited trapping efficiences (defined as the percentage of available solute which is trapped), and the presence of residual detergent even after extended dialysis or gel filtration procedures.

Advantages include the gentle nature of the procedure and the fact that solubilized membrane proteins can be reconstituted into the vesicles during the dialysis procedure.[17]

Techniques involving organic solvents have become increasingly popular. Most of these procedures involve the solubilization of the lipid in an organic solvent (e.g., ether, ethanol) which is subsequently injected into an aqueous buffer. The solvent may be removed by incubation of the buffer at a temperature above the boiling point of the organic solvent or by subsequent dialysis. A modification known as the reverse phase evaporation (REV) procedure[15] involves making an emulsion of lipid (dissolved in ether) with aqueous buffer. The organic solvent is removed under vacuum, giving rise to hydrated lipid in the form of a thick gel. This can subsequently be diluted and sized under low ($\leq$80 psi) pressure through polycarbonate filters to give LUVs of a defined size. An advantage of this technique is the high trapping efficiencies available (~35%). A general disadvantage of LUV preparations involving organic solvent concerns the differing solubilities of various species of lipid. This often necessitates different organic solvent mixtures according to the lipid employed.

Recently,[16] it has been shown that LUVs can be produced directly from MLVs by repetitively extruding MLVs through polycarbonate filters (with 100-nm pore size or less) under moderate pressures (100 to 700 psi). This technique has many advantages due to the absence of organic solvents or detergents, the straightforward and rapid protocol involved, the high trapping efficiencies available (~30%), and the generality of the technique. In particular, all lipid systems which can be dispersed in MLV form can be subsequently converted to LUV form by the extrusion procedure.

III. MEMBRANE FLUIDITY

Before discussing the properties of lipid in model systems of varying complexity, it is appropriate to indicate the problems associated with current characterizations of the properties of lipids in membranes. Chief among these is the concept of membrane fluidity, which can be most misleading. For example, it is commonly assumed that more saturated lipids or the presence of cholesterol makes membranes less "fluid". This is not necessarily the case. Strictly speaking, the fluidity parameter is the reciprocal of the membrane viscosity, which in turn is inversely proportional to the rotational and lateral diffusion rates (D_R and D_L, respectively) of membrane components.[18] Thus, a linear relation between membrane fluidity and D_R and D_L would be expected, which is not observed. Incorporation of cholesterol into phosphatidylcholine model membranes (at temperatures above the acyl chain gel to liquid crystalline transition temperature) has little or no influence on the lateral diffusion rates observed,[19,20] and can actually increase the rotational diffusion rates.[21] The major influence of cholesterol or decreased unsaturation is to increase the order in the hydrocarbon matrix. It is this increase or decrease in order, which is a measurable quantity expressed by NMR or ESR "order parameters",[22] for example, that should be correlated with such changes as decreased or increased membrane permeabilities.

IV. STRUCTURAL PROPERTIES OF LIPIDS

A. Introduction

The structural properties of phospholipids are most conveniently characterized in the simplest model systems consisting of a dispersion of the lipid in an aqueous buffer. Dispersions of individual species of liquid crystalline phospholipids of biological origin adopt either of the three structures shown in Figure 2. These include the micellar phase, which is preferred by minority lipid components such as lysophospholipids, the familiar bilayer phase, or the hexagonal H_{II} phase. The hexagonal H_{II} phase is composed of hexagonally packed cylinders of lipid where the cylinders are composed of a central aqueous channel toward

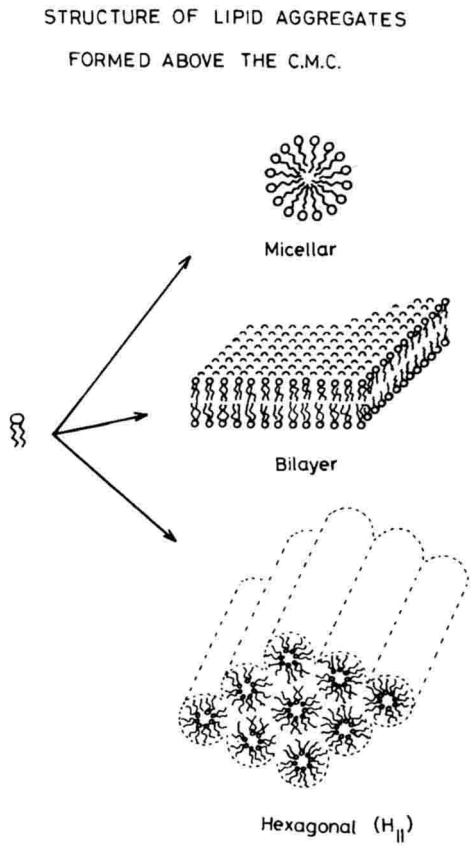

FIGURE 2. Structural preferences of liquid crystalline lipids dispersed in water at concentrations above the critical micellar concentration.

which the polar head groups are oriented. Mixed lipid systems can adopt a variety of other phases, such as those exhibiting cubic symmetry; however, the structures of these three dimensional entities are difficult to determine unequivocally. Within the context of this chapter the ability of lipids to adopt these liquid crystalline phase structures, particularly the bilayer or H_{II} arrangements or variations thereof, will be referred to as "lipid polymorphism". The literature characterizing these polymorphic properties of lipids is considerable. Here we present a synopsis of the techniques employed to monitor lipid polymorphism, analyses of the polymorphic properties of individual membrane phospholipids, and the properties of mixed lipid systems.

B. Techniques

The polymorphism of membrane lipids has been investigated by a variety of diffraction, spectroscopic, calorimetric, and other techniques. The major techniques are X-ray, NMR, and freeze-fracture; the ^{31}P NMR as well as the freeze-fracture characteristics of various lipid phases are illustrated in Figure 3. Here we briefly outline the utility and limitation of these various protocols.

X-ray and neutron diffraction are the only techniques that provide, in principle, unambiguous information on the macroscopic structure of lipid phases. A detailed discussion of their application to the study of lipid polymorphism and membrane structure is given elsewhere.[23-26] The utility of the X-ray technique for the determination of lipid phase structure

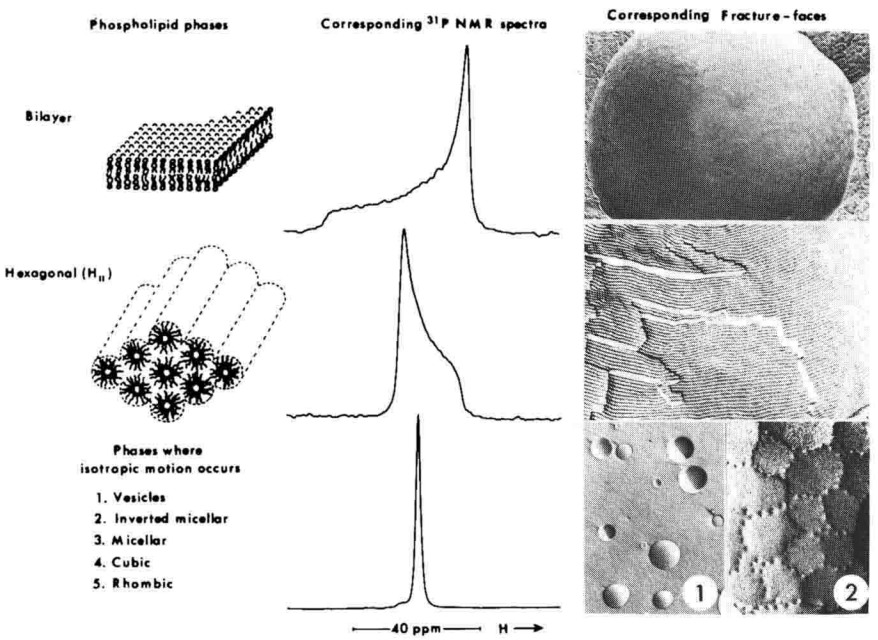

FIGURE 3. ^{31}P NMR and freeze-fracture characteristics of phospholipids in various phases. The bilayer ^{31}P NMR spectrum was obtained from aqueous dispersions of egg yolk phosphatidylcholine, and the hexagonal (H_{II}) phase spectrum from phosphatidylethanolamine (prepared from soybean phosphatidylcholine). The ^{31}P NMR spectrum representing isotropic motion was obtained from a mixture of 70 mol% soya phosphatidylethanolamine and 30% egg yolk phosphatidylcholine after heating to 90°C for 15 min. All preparations were hydrated in 10 m*M* Tris-acetic acid (pH 7.0) containing 100 m*M* NaCl and the ^{31}P NMR spectra were recorded at 30°C in the presence of proton decoupling. The freeze-fracture micrographs represent typical fracture faces obtained from bilayer and H_{II} phase systems as well as structures giving rise to isotropic motional averaging. The bilayer configuration (total erythrocyte lipids) gives rise to a smooth fracture face, whereas the hexagonal (H_{II}) configuration is characterized by ridges displaying a periodicity of 6 to 15 mm. Common conformations that give rise to isotropic motion are represented in the bottom micrograph: (1) bilayer vesicles (~100 nm diameter) of egg phosphatidylcholine prepared by extrusion techniques and (2) large lipid structures containing lipidic particles. This latter system was generated by fusing SUVs composed of egg phosphatidylethanolamine and 20 mol% egg phosphatidylserine which were prepared at pH 7 and then incubated at pH 4 for 15 min to induce fusion.

lies in the fact that lipids in phases possessing long-range order, such as the lamellar or hexagonal H_{II} phases, exhibit characteristic small-angle diffraction patterns. Lipids in a lamellar phase give rise to X-ray diffraction patterns with long spacing of the first and higher orders in the ratio 1:1/2:1/3 etc., whereas for lipids in a hexagonal phase, long spacings occur in the ratio $1:1/\sqrt{3}:1/2:1/\sqrt{7}$, etc.[23,27-31] While small-angle X-ray diffraction usually yields unambiguous information about the structure of liquid crystalline lipid phases such as the lamellar or hexagonal phases, it is generally more difficult to obtain definitive characterizations of other lipid phases such as cubic phases (although there are exceptions[32]). This is because cubic phases tend to give an insufficient number of reflections to permit unambiguous lattice assignments.[24,33,34]

Systems in which there is little long-range order exhibit diffraction profiles which are more difficult to interpret. It is difficult to detect and quantify a minority component in two or more coexisting phases, particularly when one component lacks long-range order. Further, if sophisticated detectors[35] are not employed, long exposure times (hours to days) are required; this is a problem for labile lipids. In addition, sample preparation is generally not as straightforward as with other spectroscopic or calorimetric techniques. In order to maximize signal intensities it is advantageous to prepare the sample as oriented, closely opposed multilayers

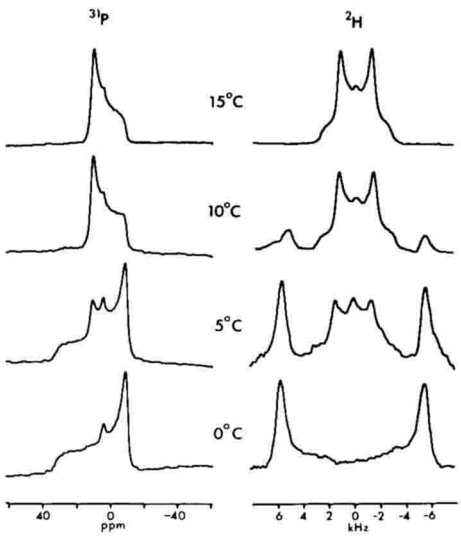

FIGURE 4. ^{31}P and ^{2}H NMR spectra as a function of temperature of fully hydrated dioleoylphosphatidylethanolamine (DOPE), which is ^{2}H labeled at the C_{11} position of the acyl chains ([$C_{11}-{}^{2}H_2$]DOPE). The ^{31}P NMR spectra were obtained at 81.0 MHz in the presence of proton decoupling, whereas the ^{2}H NMR spectra were obtained at 30.4 MHz. (From Tilcock, C. P. S. et al., *Biochemistry*, 21, 4596, 1982. With permission.)

(where the phase properties allow); while this is a relatively straightforward procedure for pure lipid systems, it is not so readily achieved with biological membranes.[24] These disadvantages aside, X-ray continues to be the method of choice for the unambiguous determination of lipid phase structure. Studies with neutron diffraction have been limited by the somewhat expensive hardware involved.

More recently, the techniques of ^{31}P NMR and ^{2}H NMR have been applied to the study of lipid polymorphism. Detection of a bilayer-H_{II} transition in DOPE ^{2}H labeled in the acyl chains ([C_{11}-$^{2}H_2$]-DOPE) by ^{31}P and ^{2}H NMR is illustrated in Figure 4. ^{31}P NMR has proven particularly useful and relies on the following factors. The electron distribution surrounding a phospholipid phosphorus nucleus is anisotropic and thus in a magnetic field the phosphorus experiences an orientation-dependent "shielding" termed the "chemical shift anisotropy" (CSA). For a dry lipid powder the resulting ^{31}P NMR spectrum represents a superposition of resonances from all orientations. For lipids in large (radius $\geq$200 nm) liquid crystalline systems the CSA is partially averaged on the NMR timescale by the rotational axial motion of the molecule. In the presence of proton decoupling, this results in a characteristic asymmetric ^{31}P NMR lineshape with a low-field shoulder and high-field peak, exhibiting an effective CSA (measured from the low field shoulder to the high field peak) of approximately -40 to -50 ppm, depending upon the lipid species, temperature, or other factors.[8,36-39] That this lineshape also represents a superposition over all orientations may be seen on examining the ^{31}P NMR spectrum from bilayers oriented between glass plates.[39] Different orientations of the plates with respect to the applied field results in individual narrow resonances with an angular-dependent chemical shift. The resonance position of the high-field peak of the unoriented system corresponds to an orientation of the lipids with their long axes normal to the applied field, while the resonance position of the shoulder corresponds to an orientation parallel to the field. The asymmetry of the lineshape arising from the unoriented system arises from the fact that there is a greater probability of finding a lipid with its long axis perpendicular, rather than parallel to, the applied field.

For a lipid in the hexagonal H_{II} phase additional motional averaging is experienced due to diffusion of the lipids around the cylinders. This results in a two-fold reduction in the effective CSA and a reversed asymmetry.[8,36,38] In particular, there is now a smaller probability of finding a cylinder of lipid parallel to the field (in which all the long axes of the lipid will be perpendicular to the field) than finding a cylinder of lipid perpendicular to the field. Thus, the relative intensities of the low- and high-field shoulders are reversed compared to the lamellar system. It should be recognized that if the rate of diffusion of the lipid molecules around the cylinder is slow relative to the spectroscopic timescale ($\sim 10^{-5}$ sec) then no additional averaging effects will be observed. This is part of the reason why ESR spin-probes are not well suited to monitoring lamellar-H_{II} transitions.[40] In systems where molecular reorientation occurs over all possible orientations (isotropic motion) in times on the order of the NMR timescale or less, motional averaging results in a single narrow ^{31}P NMR resonance. This occurs for lipids in small vesicles, micelles, inverted micelles, or other phases such as the cubic.

The reliability of ^{31}P NMR phase identifications has been questioned as theoretical considerations show that changes in the conformation of the phosphoryl segment of the lipid headgroups could result in the observed spectral differences without any change in phase structure.[41,42] However, numerous experiments have failed to demonstrate any such conformational changes, and the assignments of phase structure based on ^{31}P NMR data have been fully corroborated by X-ray diffraction for various phosphodiester lipids.[31,34,43-45] This supports the general utility of the ^{31}P NMR method. It should, however, be pointed out that ^{31}P NMR (and ^{2}H NMR) does not provide structural information per se, but tells of the motional averaging properties of the ensemble. ^{31}P and ^{2}H NMR are thus used in an extrapolative manner, the interpretation being based on prior structural assignments by X-ray or neutron diffraction.

An advantage of ^{31}P NMR over X-ray diffraction is that it is technically easier to apply, particularly to complex biological systems. Since ^{31}P is the naturally occurring isotope, no isotopic enrichment is required; as there is only one phosphorus per phospholipid, interpretation is straightforward. The principal disadvantages of the technique are that it can only be used for lipids that contain phosphorus and that it is generally not possible to examine the phase behavior of a particular lipid within a mixture of phospholipids. Exceptions include situations where the lipid of interest has a considerably different CSA[45,46] or a different spin-lattice relaxation time.[47] Finally, compared to X-ray, ^{31}P NMR phase determinations require a large amount of material (20 μmol phospholipid or more) if excessive accumulation times are to be avoided.

^{2}H NMR techniques can also be employed to identify lipid phase structure (Figure 4) Such determinations are based upon very similar principles to ^{31}P NMR. In particular, the quadrupolar moment of each deuterium gives rise to a doublet, whose splitting is dependent on the orientation of the magnetic field with respect to the ^{2}H nucleus. In non-oriented lamellar systems this gives rise to a "powder pattern" where the separation between the two major peaks is known as the quadrupolar splitting. In the absence of additional local motion, lateral diffusion around the cylinders of H_{II} phase systems results in a reduction in the quadrupole splitting by a factor of 2, whereas for isotropic motion, the ^{2}H NMR spectrum consists of a single line. Clearly, for studies of lipid polymorphism, it is possible to have only one lipid in a multicomponent system labeled with deuterium and thus be able to examine its particular phase behavior. The primary disadvantage of the application of ^{2}H NMR to the study of lipid polymorphism and membranes in general is the requirement for organic syntheses of specifically deuterated molecules; however, methods for the incorporation of deuterium at many positions within a lipid molecule are now well established.[22,63] In addition, the development of "de-Paking" techniques for the study of ^{2}H NMR spectra avoids some of these difficulties.[48] The application of ^{2}H NMR to the study of lipid and