BIOTECHNOLOGICAL APPLICATIONS OF LIPID MICROSTRUCTURES

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PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Based on the Proceedings of the Workshop on Technological Applications of Phospholipid Bilayers, Vesicles, and Thin films (1986: Tenerife, Canary Islands)

Biotechnological applications of lipid microstructures / edited by Bruce Paul Gaber, Joel M. Schnur, and Dennis Chapman.

p. cm. – (Advances in experimental medicine and biology; v. 238)

Based on the Proceedings of the Workshop on Technological Applications of Phospholipid Bilayers, Vesicles, and Thin Films, held January 5–9, 1986, in Tenerife, Canary Islands; and the Workshop on Biotechnological Applications of Membrane Studies, held October 18–22, 1987, in conjunction with the Second World Basque Conference in San Sebastian, Spain.

Bibliography: p.

Includes index.

ISBN 0-306-43014-2

1. Bilayer lipid membranes – Biotechnology – Congresses. 2. Lipid membranes – Biotechnology – Congresses. I. Gaber, Bruce Paul. II. Schnur, Joel M. III. Chapman, Dennis, date. IV. Biotechnological Applications of Membranes Studies Second World Basque Conference (1987: San Sebastian, Spain) V. Title. VI. Series.

TP248.25.B53S95 1986 88-8304

660'.6 – dc19 CIP

Based on the Proceedings of the Workshop on Technological Applications of Phospholipid Bilayers, Vesicles, and Thin Films, held January 5–9, 1986, in Tenerife, Canary Islands; and the Workshop on Biotechnological Applications of Membrane Studies, held October 18–22, 1987, in conjunction with the Second World Basque Conference in San Sebastian, Spain

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

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TECHNOLOGICAL APPLICATIONS OF PHOSPHOLIPID BILAYERS, VESICLES AND THIN FILMS

Puerto de la Cruz
Tenerife, Canary Islands
5-9 January 1986

Organizing Committee

Bruce Paul Gaber, Washington, D.C.
Joel M. Schnur, Washington, D.C.
Dennis Chapman, London, U.K.
James A. Hayward, Stony Brook, N.Y.

BIOTECHNOLOGICAL APPLICATIONS OF MEMBRANE STUDIES II WORLD BASQUE CONFERENCE

Donostia-San Sabastian
Basque Country, Spain
18-22 October 1987

Organizing Committee

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PREFACE

In the twenty years since Bangham first described the model membrane system which he named "liposomes", a generation of scientists have explored the properties of lipid-based microstructures. Liposomes of all sizes, tubular and helical structures, and self-assembled lipid films have been prepared and studied in detail. Many of the advances in the basic research have led to significant technological applications. Lipid microstructure research has begun to mature and it is an appropriate time for an in-depth look at the biotechnological applications, both achieved and potential.

As a forum for active discussions within this growing field, two Workshops were organized: "Technological Applications of Phospholipid Bilayers, Vesicles and Thin Films", held in Puerto de la Cruz, Tenerife, Canary Islands; and "Biotechnological Applications of Membrane Studies", held in Donostia-San Sabastian, Basque Country, Spain. The organizers of these Workshops believe that development of lipid self-assembly into a technological discipline requires significant interaction across traditional scientific boundaries. Thus the Workshops gathered an eclectic group of colleagues whose interests ranged from basic research into structure, interactions and stabilization of biomembranes to applications of lipid microstructures such as artificial cells, diagnostic reagents, energy transfer systems, and biosensors.

This book, the tangible product of the Workshops, consists of invited contributions from participants in both meetings. The intangible results -- and perhaps those most important in the long run -- were the spirited exchanges of ideas which occurred throughout the meetings and the new collaborations and research which followed. The synergy that the organizers of these Workshops had hoped to catalyze was truly realized.

Both Workshops were possible only with the support of sponsors willing to underwrite meetings in a new and evolving field. The Workshop in Tenerife was supported by the Office of Naval Research, London (Dr. T. Rozelle); the U. S. Army Research Standardization Group (Dr. D. Squires); the U.S. Air Force European Office of Aerospace Research and Development (Dr. R. Drawbaugh); and Smith, Kline, and French Laboratories (Dr. G. Poste). The Donostia-San Sabastian Workshop, splendidly organized by Professor Felix Goñi, was held under the auspices, and with the generous support, of the Second Basque World Conference (Sr. L. Gurruchaga).

Our special thanks go to Ms. Helen Beakley whose service has been invaluable both in the coordination of the Tenerife Workshop and as the editorial assistant for this volume.

For the editors,

Bruce Paul Gaber

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MOLECULAR MODELING OF THE PHOSPHOLIPID BILAYER

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INTRODUCTION

We have developed computer graphics models of the phospholipid bilayer which are based upon published X-ray crystallographic data. We believe that these depictions convey significantly more information about the structure and chemistry of lipid molecules and bilayers than do the iconographic or schematic representations of the past. These graphic models, beyond their utility for visualization of structure, provide a starting point for subsequent higher level molecular modeling (molecular mechanics, graphics and dynamics) of systems including polymerizable lipids, bilayer/small molecule complexes and lipid/protein interactions.

Here we discuss the fundamental methodology of the development of the models and their computer graphics realization; the application of the model to analysis of the structure of dimyristoylphosphatidylcholine (DMPC); the extension of the technique to modeling a diacetylenic lipid; and the use of the model to explore the interaction of trehalose with the phospholipid bilayer.

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A word of caution -- we are dealing with idealized models derived from lipid crystals. The models are simple, static representations of single component lipid bilayers. The models do not yet account for chain fluidity nor do they include other phospholipids, cholesterol or proteins. Nonetheless, the models presented here are instructive into the nature of lipid structure and organization.

METHODOLOGY

Our modeling begins with construction of a database consisting of the coordinates of lipids for which crystallographic data are available. Working with the coordinates for the lipid molecules constituting the crystallographic unit cell and knowledge of the space group in which the lipid crystallizes, it is possible to transform the initial fractional coordinates into an orthogonalized set of coordinates representative of a bilayer. Lipids appear to be rather more recalcitrant than proteins to form usable crystals, but the determined efforts of several laboratories (Pascher et al., 1987 and references therein) have resulted in a range of structures sufficient for our initial purposes.

Dimyristoylphosphatidylcholine crystallizes in a unit cell composed of four molecules organized as tail-to-tail pairs. Each pair consists of two molecules (type A and type B) which differ primarily in the conformation of the head group (Pearson and Pascher, 1979). The space group P21 defines the symmetry operations that replicate the molecules of the unit cell into an entire crystal. We have constructed a model of DMPC consisting of 36 lipid molecules arranged 6-by-6 in each of the upper and lower monolayers. The size of the model is limited only by computational convenience. In the DMPC model this is about 3400 atoms, roughly equivalent to a protein of 44200 daltons. The data file is arranged in Protein Data Bank format with each lipid molecule assigned an unique residue number. Thus any particular molecule or cluster of molecules may be selected for closer study.

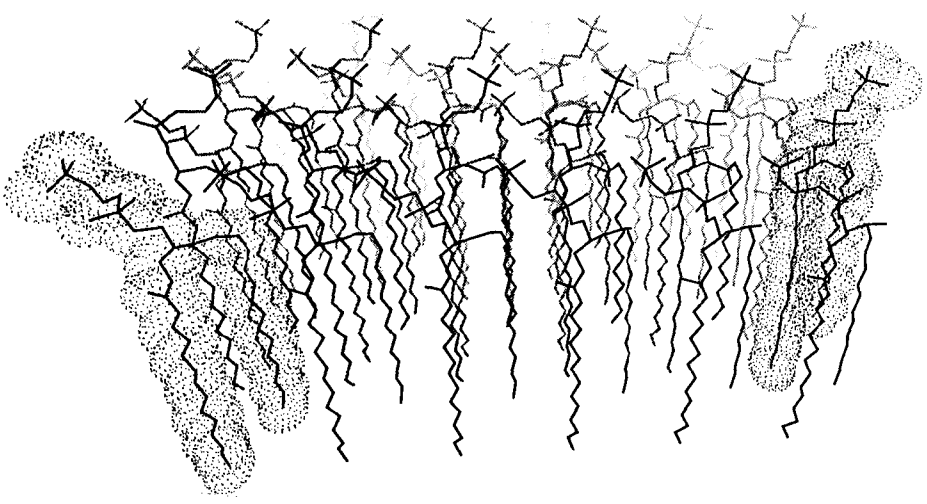

Fig. 1. Model of a 36 molecule portion of the DMPC monolayer.

For interactive molecular graphics of the lipid models, we use an Evans & Sutherland PS 330 or a Silicon Graphics Iris system. These devices permit direct, real-time manipulations of the model such as rotation, translation and scaling. Solvent-accessible and van der Waals surfaces are easily computed. The graphical modeling software includes FRODO (Jones, 1978) and MIDAS (Jarvis et al., 1985). Black and white figures are generated by post-processing the computer's color image using the software PostScript. A DMPC monolayer generated by this procedure is shown in figure 1.

High resolution, space-filling, raster images are realized using the software package **Spock**, written by Dr. R. Brown of the Naval Research Laboratory. **Spock** was designed specifically for lipid modeling and has an extended data structure which permits files of any size to be easily manipulated. This capability is particularly valuable for handling very large lipid arrays. The program permits choices of atom color, size and shading, and an option for stereo viewing. Although developing a very high resolution image with **Spock** is computationally intensive, the program has a preview feature which permits rapid on-screen manipulation of skeletal and ball-and-stick models. Once a desired view is chosen, it can be easily converted to a full space-filling image.

FUNDAMENTALS OF PHOSPHOLIPID STRUCTURE

The DMPC Molecule

Most of our detailed knowledge of the structure of DMPC was derived from the detailed analysis by Hauser et al. (1981) of the crystallography of Pearson and Pascher (1979). Many of the observations here are based on their work.

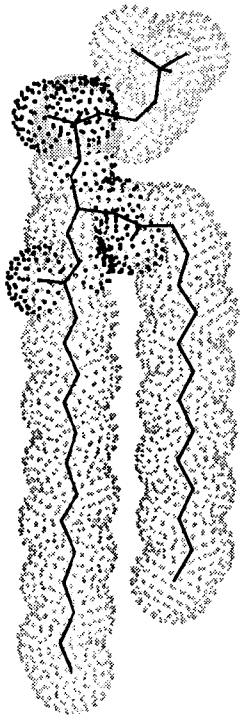

Fig. 2. Structure of the DMPC type B conformer depicted as a skeletal model (heavy line) with a superimposed van der Waals surface. Light dots denote carbon; heavy dots outline oxygen; solid gray represents phosphorus; nitrogen is not visible.

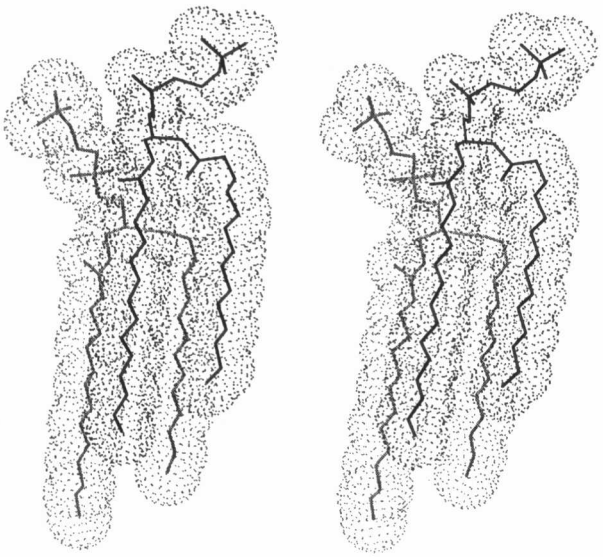

Fig. 3. Stereo pair depicting type B (dark lines) and type A (light lines) conformers of DMPC.

The dominant structural element of the DMPC molecule (figure 2) is the asymmetric arrangement of the acyl chains. Chain sn-1 forms an all-**trans** zig-zag structure which extends the three carbons of the glycerol. However, the sn-2

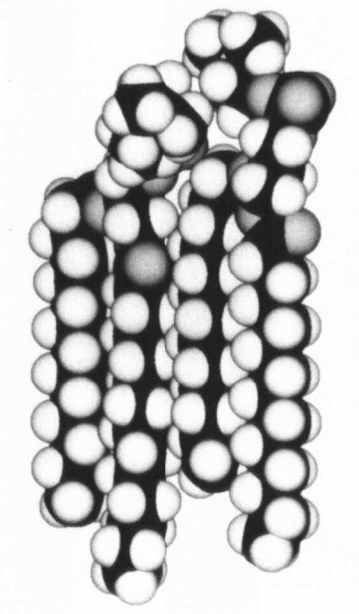

Fig. 4. Space-filling model of type A and type B conformers of DMPC generated using SPOCK.

chain extends normal to the glycerol moiety for two methylene units and then turns and runs parallel to chain sn-1. The result of the chain asymmetry is an offset of approximately three methylene units (3.7 Å) between chains sn-1 and sn-2. This conformational non-identity of chains sn-1 and sn-2 appears to be a fundamental element of phospholipid structure. This was first suggested by Seelig and Seelig (1975) on the basis of deuterium NMR studies and later implied by Raman spectroscopy of selectively deuterated lipids (Gaber et al. (1978)).

The DMPC crystal contains two conformationally distinct DMPC molecules, characterized primarily by differences in the head group (figure 3). For the type A conformer, the vector connecting the phosphorus and nitrogen lies at an inclination of about 17 degrees relative to the bilayer normal, while in the type B molecule the P-N vector is at 27 degrees. Further, within the bilayer, the conformers are offset with respect to one another by about 2.5 Å in the direction of the bilayer normal; the type A molecule is displaced toward the bilayer midplane. The result is an alternating pattern in which the type B headgroups are highly exposed at the bilayer surface, while type A headgroups are almost buried. A space-filling model (generated using **Spock**) emphasizes the close packing of the two molecules (figure 4).

Organization of the Bilayer

The extremely compact structure of the DMPC bilayer can be appreciated from the spacing-filling side views in figures 5 and 6. A 12 degree tilt of the chains is evident when the bilayer is viewed along the X-axis (figure 5). This slight tilt is just sufficient to allow the cross-sectional area of the chains (38 $Å^2$) to be accommodated under the head group. The carbonyl oxygens of both chains sn-1 and sn-2 of type B, and of chain sn-2 of type A are in the head group region, but the carbonyl oxygen of chain sn-1 of the more deeply penetrating type A is very nearly within the hydrophobic region of the bilayer. The model demonstrates that the bilayer chains are interdigitated only at the bilayer midplane. Interdigitation is also seen when the bilayer is rotated 90 degrees (figure 6).

When viewed almost directly down onto the headgroup region (figure 7), the orderly arrangement and tight packing of the bilayer is most striking. Rows of type A molecules alternate with type B conformers and considerable space exists between successive tetrads of type B headgroups. This space is occupied by bound water.

Water and the Bilayer

Solvent of crystallization also can be included in the bilayer model. Four waters of crystallization are associated with each pair of lipid molecules. Of these, three are hydrogen bonded directly to the lipid headgroups. Two of these are involved in bridging the head groups of type A to type B. The third water links a head group to a fourth water, which is in turn hydrogen bonded to the adjacent bilayer. This elaborate hydrogen bonding network plays a role in the stabilization of the bilayer (Pearson and Pascher, 1979).

A useful way to depict the interaction of water with the bilayer is to generate a water-accessible surface. This procedure uses an algorithm devised by Connolly (Langridge et al., 1981) in which a probe sphere with the radius of a solvent molecule is computationally "rolled" over the molecular van der Waals surface. The water accessible surface is generated from the points of contact of the probe sphere and the van der Waals surface. Via the application of this procedure to the bilayer headgroup region, a surface is generated which reveals two potential water accessible regions of the DMPC bilayer (figure 8). The first consists of a series of deep holes in the surface, while the second is a set of pockets or ridges that appear to be nearer to the surface of the bilayer.

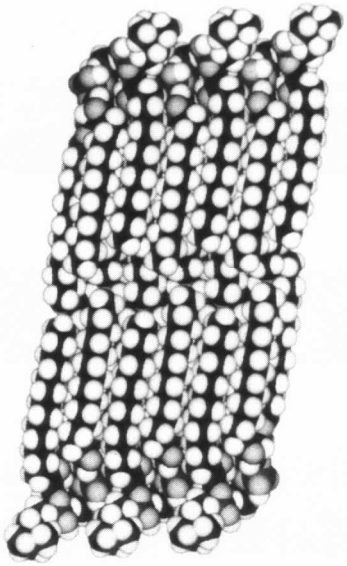

Fig. 5. Space-filling side view (along x-axis) of the DMPC bilayer.

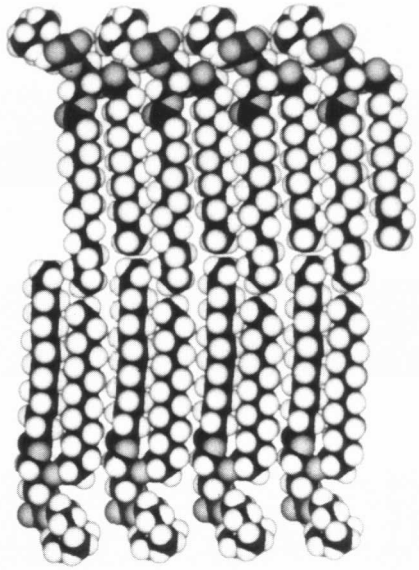

Fig. 6. Space-filling side view (along Y-axis) of the DMPC bilayer.

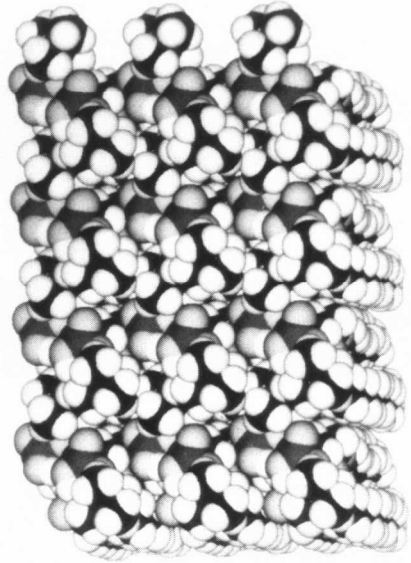

Fig. 7. Headgroup region of the DMPC bilayer.

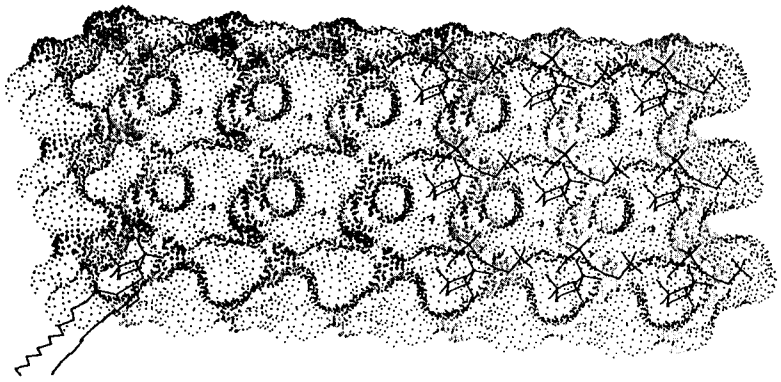

Fig. 8-a. Water accessible surface viewed down onto the headgroup region of the DMPC bilayer showing the locations of pairs of tightly bound waters of crystallization.

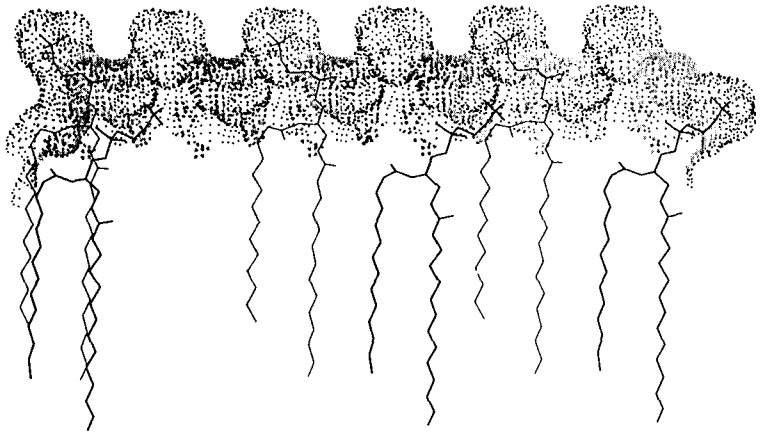

Fig. 8-b. Water accessible surface as in figure 8-a, viewed perpendicular to the acyl chains.

APPLICATIONS OF THE BILAYER MODEL

Diacetylenic Lipids: A Preliminary Model

The phospholipid data base and modeling procedures described here can be used as an aid to understanding molecules for which structural data is absent or incomplete. The diacetylenic phosphatidylcholine $DC_{89}PC$, described in detail in this volume by Rudolph et al., is one such molecule. The nomenclature $DC_{mn}PC$ defines the number of methylenes groups preceding (m) and following (n) the diacetylenic moiety.

To construct a preliminary model of $DC_{89}PC$ (figure 9), we have assumed that the headgroup of the molecule shares structural features in common with other phosphatidylcholines. Accordingly, we have adopted the headgroups and chain conformation from the Pearson and Pascher (1979) DMPC crystal structure. Working at a graphics workstation, we attached all-trans m,n-diacetylenic acyl chains to the A- and B-type headgroups. The chain conformations were altered

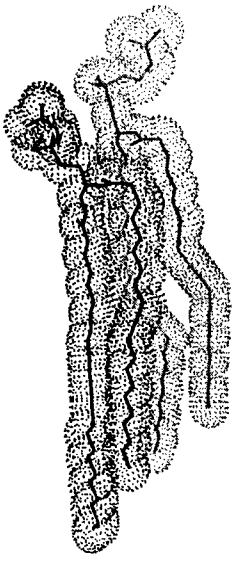

Fig. 9. Hypothetical model of a pair of $DC_{89}PC$ diacetylenic lipids based upon the crystal structure of DMPC. The model has been interactively modified for close packing.

to relieve close contacts. The chains of the resulting structures are relatively close-packed within each molecule. The two molecules also are closely packed, especially above the diacetylenic region. Due to the close contacts of the acyl chains, the AB pair is no longer an element of a crystallographic unit cell; i.e., it cannot be translated to form a close-packed monolayer, as can the AB pair of DMPC. However, the model is a useful tool and a reasonable starting point for energy minimization.

An interesting situation develops when we attempt to model the compound $DC_{98}PC$, the positional isomer of $DC_{89}PC$. The molecule is obtained by moving the diacetylenic moiety down the acyl chain by one methylene unit. A preliminary model (figure 10, left) shows the chains are splayed. To achieve close-packing of the chains in $DC_{98}PC$, we have introduced a g^- (300^o) torsion about the bond C16-C17, and a g^+ (56^o) torsion about the bond C115-C116, in chain sn-1. This "extended kink" is similar to an ordinary kink (g^+tg^- or g^-tg^+) in an alkyl chain; the direction of the chain is maintained on either side of the kink ($^-$_). The effect on the sn-1 chain of the $DC_{98}PC$ lipid is to bring it closer to the sn-2 chain, creating a more compact unit (figure 10, right).

This particular model has no experimental support, but it does provide testable predictions. First, Raman spectroscopy of the longitudinal acoustic modes of the m-odd series lipids should reflect the presence of shorter all-trans methylene segments. Second, the d-spacing of m-odd lipids should be slightly less than the isomeric m-even lipids having the same number of methylenes. An m-odd:n-even alternation in Tm has been noted (Rudolph et al., 1988) and is consistent with a difference in chain packing between the two isomers.

Bilayer-Disaccharide Interactions

A variety of biological organisms such as plant seeds and yeast can, during certain stages of their life cycles, withstand extraordinary levels of dehydration (Leopold, 1986). This phenomenon, often called "anhydrobiosis", appears to be intimately related to the stabilization of an organism's membranes by specific