

Mini-Review

A New Look at Lipid-Membrane Structure in Relation to Drug Research

Ole G. Mouritsen^{1,3} and Kent Jørgensen²

Received May 7, 1998; accepted June 30, 1998

Lipid-bilayer membranes are key objects in drug research in relation to (i) interaction of drugs with membrane-bound receptors, (ii) drug targeting, penetration, and permeation of cell membranes, and (iii) use of liposomes in micro-encapsulation technologies for drug delivery. Rational design of new drugs and drug-delivery systems therefore requires insight into the physical properties of lipid-bilayer membranes. This mini-review provides a perspective on the current view of lipid-bilayer structure and dynamics based on information obtained from a variety of recent experimental and theoretical studies. Special attention is paid to trans-bilayer structure, lateral molecular organization of the lipid bilayer, lipid-mediated protein assembly, and lipid-bilayer permeability. It is argued that lipids play a major role in lipid membrane-organization and functionality.

KEY WORDS: lipid bilayer; molecular organization; phase transitions; permeability; lipid-protein interaction.

INTRODUCTION

In aqueous environments, lipid molecules self-assemble spontaneously into various molecular aggregates of which the lipid bilayer is the most important structure in relation to biological systems. Phospholipid bilayers constitute the core of biological membranes. Simple lipid-bilayer systems, such as liposomes, are therefore useful models of biomembranes. Furthermore, liposomes are interesting for pharmaceutical purposes either as drug-delivery systems or as assay for studying drug-membrane interactions. The current conventional perception of lipid bilayers and lipid-bilayer structure, still prevalent in many modern textbooks, is strongly influenced by the fluid-mosaic model of cell membranes advanced more than 25 years ago by Singer and Nicolson (1). Within this model, the lipid-bilayer component of cell membranes is considered a two-dimensional fluid matrix in which the membrane proteins are embedded. Apart from being a bimolecular sheet, providing a suitable fluid solvent that allows for the necessary protein mobility, the lipid bilayer is imparted with little structural detail within the Singer-Nicolson model.

Research in the physical properties of lipid membranes (2,3,4) has however shown that lipid bilayers are highly structured fluids both in space and time. Furthermore, bilayers display correlated modes that are intricately related to the inherent liquid-crystalline character of the bilayers and to the fact that they are many-particle systems sustaining cooperative and colligative phenomena (5). Moreover, evidence is accumulating that this lipid-bilayer structure and dynamics play an important role for membrane functionality (16).

In order to appreciate and understand which aspects of lipid-bilayer structure are important for certain pharmaceutical purposes it is necessary to clarify what might be the relevant length scales and time scales. At the present time, it is in most cases not possible to assign actual numbers to these scales. The present paper presents a qualitative framework within which one might eventually determine these scales quantitatively. A couple of specific examples are in order. In the case of small molecular compounds or ions which permeate or partition into bilayer membranes, molecular-scale properties of the hydration barrier and the trans-bilayer molecular profile are highly relevant in addition to the small-scale lateral molecular organization of the bilayer at the place where the permeant impinges on the membrane. In the case of enzymes, peripheral proteins, hormones, and drugs interacting with the extrinsic parts of membrane-bound receptors, the scale in question is of the order of the size of the foreign compound. In the case of amphiphilic peptide drugs associating with the membrane surface, the nature of the hydrophilic-hydrophobic membrane interface and its structural heterogeneity is at issue, and for fully or partly membrane-spanning peptides the trans-bilayer structure also comes into play. In this case the spatial scale of interest is that of the bilayer thickness. When it comes to the use of lipid-bilayer systems as drug carriers, e.g., in the form of liposomes and

¹ Department of Chemistry, Technical University of Denmark, Building 206, DK-2800 Lyngby, Denmark.

² Department of Pharmaceutics, The Royal Danish School of Pharmacy, DK-2100 Copenhagen Ø, Denmark.

³ To whom correspondence should be addressed. (e-mail: ogm@kemi.dtu.dk; WWW: <http://www.fki.dtu.dk>)

ABBREVIATIONS: DC_nPC, diacyl-glycero PC with *n* carbons in each chain; DOPC, di-oleyl PC; ESR, electron spin resonance; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEG, poly-ethylene glycol; PS, phosphatidylserine.

transfersomes, the whole size of the liposomal object plays a role, specifically its mechanical properties and the shape and shape deformations of the liposome. Finally, in the case of drugs whose action is lipid mediated, scales from the size of an individual lipid molecule to that of a sub-micron-size membrane domain within which the drug diffuses can be relevant. In all cases, the scales considered should be considered as time-dependent in the sense that the various aspects of the bilayer structure each have their characteristic time scale. Therefore, the events taking place should be considered with reference to the relevant time scales.

Advances in our understanding of lipid-membrane structure and dynamics have built upon the use of a host of powerful physical experimental techniques as well as model calculations (2,4). Experimental techniques like magnetic resonance spectroscopy, fluorescence spectroscopy, various types of microscopies, micromechanics, ultra-sensitive surface-probe techniques, as well as scattering and diffraction have provided information in the range from atomic scales to the size of cells. Parallel use of theoretical methodologies, like molecular dynamics and computer-simulation techniques, have added to this information and helped to interpret some of the more subtle experimental observations as well as been instrumental for designing more well-focused experimental approaches.

In the present mini-review we shall provide a perspective of the picture of lipid-bilayer structure that in recent years has emerged from these endeavours. The review is in no way intended to be an exhaustive account, nor does it give full credit to the very substantial and rapidly expanding literature in this field. References are often only given to recent key reviews and books in which the interested reader can find proper references to the original papers in more specialized journals. The review is organized in two parts. In the first part, general physical properties of lipid-bilayer structure and molecular self-assembly and organization of bilayer membranes are described. In the second part, a number of pharmaceutical relevant membrane phenomena are considered as case studies in relation to the physical properties of membranes. These phenomena include membrane permeability, liposomal drug delivery, enzyme/protein/receptor function, and membranes as targets for drugs.

LIPID-BILAYER STRUCTURE AND STABILITY

Transverse Structure of Lipid Bilayers

A composite presentation of a typical trans-bilayer profile is given in Fig. 1 which shows a collection of data obtained from both experimental and theoretical studies. In Fig. 1a are given results from a molecular dynamics calculation (7). This picture, which is a snapshot corresponding to a specific time, reveals the distribution of the various parts of the lipid and water molecules. In Fig. 1b are shown results from X-ray scattering of the time-averaged probability of finding the different parts of the molecules across the lipid bilayer (8,9). The profiles in Fig. 1b correspond to electron-density maps. Taken together, the two representations of the trans-bilayer structure in Fig. 1 illustrate both static and dynamic aspects of the trans-bilayer membrane structure. In particular they show that the bilayer is a very stratified composite consisting of a layer [1] of perturbed water, a chemical heterogeneous layer [2] of hydrophilic/hydrophobic material including bound water, lipid-polar-head

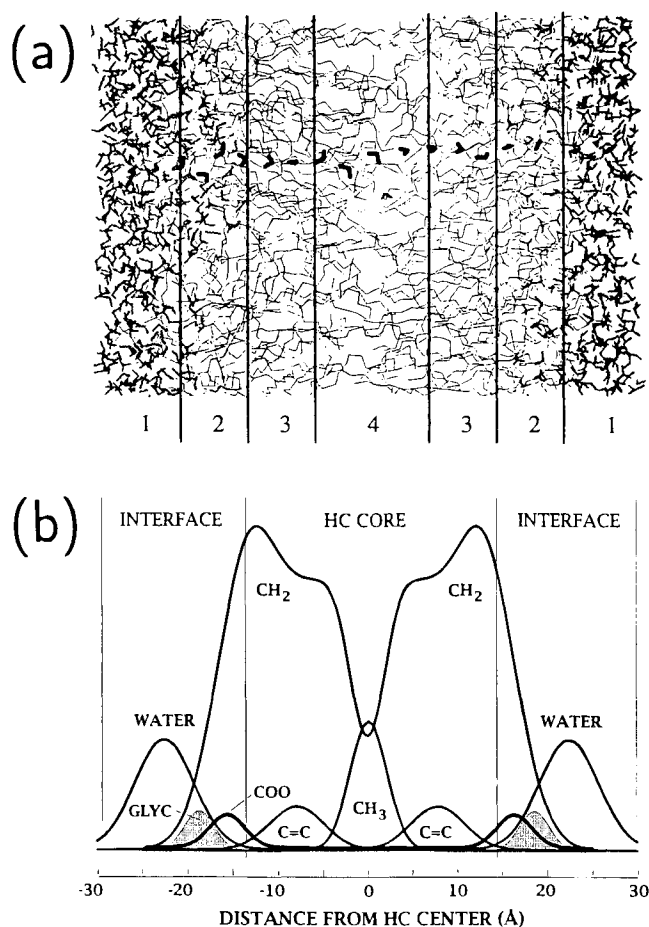


Fig. 1. Trans-bilayer structural profiles. (a) snapshot of microscopic molecular conformation as obtained from a molecular dynamics calculation on DC₁₆PC bilayers. (From Ref. (7) with permission). (b) electron-density profile as obtained from X-ray scattering on DOPC bilayers. (From Ref. (8) with permission).

groups, and parts of the upper end of the acyl chains, a soft-polymer-like layer [3] consisting of conformationally ordered acyl-chain segments, and a layer [4] of conformationally disordered acyl-chain segments of a structure similar to liquid decane (7). A detailed comparison between a calculated and experimentally determined trans-bilayer profile has recently been presented in the case of unsaturated fatty acid chains which are important for biological membranes (10).

In a pharmaceutical context, the following properties of this trans-bilayer structure are of importance. Firstly, the lipid bilayer is associated with a layer of so-called perturbed water with polarization properties that are very different from ordinary water (e.g., as reflected in a dielectric constant that is an order of magnitude lower than that of bulk water). The capability of this layer of water to form hydrogen bonds with approaching drugs is dramatically reduced. Secondly, the hydrophilic-hydrophobic interface is smeared out providing a thick layer (layer 2) that is prone for non-covalent bonding with drugs like amphiphilic peptides. This layer is likely to play an important role for folding and unfolding of peptide drugs upon binding and subsequent penetration (9). Layers [1] and [2] present the critical dehydration barrier for ions and solutes that partition into and dissolve in the membrane (11). It is remarkable that

these layers together constitute about half of the lipid-bilayer thickness. Finally, the structural differences between layers [3] and [4] play an important role for the diffusional characteristics of permeants and the orientation of anisotropic drug molecules once penetrated into the hydrophobic core of the bilayer (12).

Lateral Bilayer Structure and Membrane Domains

Whereas the trans-bilayer stratification and heterogeneity in some way qualitatively is an obvious consequence of the bi-molecular sandwich structure of lipid bilayers, the lateral organization is much less obvious and therefore also much less appreciated (13,14). Nevertheless, lateral lipid-bilayer heterogeneity is a fundamental consequence of the many-particle character of the lipid-bilayer assembly (5,14,15). The molecular interactions support collective and cooperative phenomena that laterally lead to static and dynamic structure on length scales ranging from the single molecule to the entire system, depending on bilayer composition, thermodynamic conditions such as temperature and pressure, as well as influence of external factors like pH and ionic strength. Examples of static lateral organization include phase separation and lipid-microdomain organization due to coupling to extramembranous moieties (16,17).

The type of dynamic small-scale lateral organization one should expect to encounter in lipid bilayers is illustrated in Fig. 2 which shows the structural dynamic heterogeneity that is respectively due to density fluctuations close to phase transitions (Fig. 2a) and compositional fluctuations in binary lipid mixtures (Fig. 2b). In both cases, the lipid domains formed are dynamic, but they are characterized by a certain average length scale that depends on temperature, composition, and the type of lipid bilayer in question. A typical length scale is in the range of 1–100 nm. An example of the non-equilibrium lipid-domain organization that can arise in phase-coexistence regions is shown in Fig. 2c. The existence of lateral domain organization in the nanometer range predicted by computer-simulation pictures like in Fig. 2 has been verified by a number of direct and indirect experimental techniques, including fluorescence spectroscopy (18,19,20,21), neutron scattering (22), and atomic-force microscopy (22,23) (for a recent review, see Ref. (14)).

In relation to membrane function in a pharmaceutical context, the presence of a heterogeneous lateral organization may have a number of important consequences of which we list a few: a) The structural molecular packing disorder implied by domains patterns and in particular the interfaces bounding the domains will enhance penetration and insertion of foreign molecules (like peptides, proteins, and drugs) into the bilayer and generally increase bilayer permeability (24). b) The dynamic heterogeneity implies softening of the bilayer mechanical properties, e.g., the lateral compressibility and the bending rigidity (25), since in-plane organization and fluctuations are coupled to the local membrane curvature. This softening in turn leads to changes in membrane capacities like fusigenicity, vesiculation and cytolysis, as well as the interaction with other membranes and surfaces (26). The functioning of transfersomes as controlled by their mechanical properties is one important example of this behavior (27). c) The formation of lipid domains of a particular composition and structure implies differentiation and compartmentalization of the lipid bilayer which control the association and binding of peripheral (e.g., charged) macromolecules and enzymes. Examples include the binding and/or activity of e.g.,

cytochrome c (28), protein kinase C (29,30,31), MARCKS (myristoylated alanine-rich C kinase substrate) (32), and phospholipases (33,34,35,36). d) The domain organization and the connectivity properties of the different membrane regions have consequences for the diffusional properties of membrane-bound molecules, such as enzymes and receptors, and may hence control the kinetics and reaction yields of the associated chemical reactions. Possible scenarios include accumulation and colocalization of receptors and drugs in the same (small and specialized) membrane compartments via the cooperative domain-organization processes (37) and specific percolation events (38). e) The physico-chemical effect of an applied drug may itself have an influence on the lateral organization of the lipid membrane (39), as illustrated in Fig. 2d.

Curvature Stress in Lipid Bilayers

Not all membrane lipids support bilayers. In fact a large amount of common phospholipids, like phosphatidylethanolamines, show propensity for forming non-lamellar phases with curved interfaces, such as hexagonal and cubic phases (39). These non-lamellar phases may not necessarily manifest themselves globally, but instead associate the bilayer with an intrinsic curvature-stress field (40) which locally can be expressed and released in connection with fusion, binding of peripheral proteins, insertion of peptides, or translocation events (41). Non-lamellar-forming lipids can play a particular role in modulating lipid-protein interaction and for triggering protein or membrane-channel function (42). Some of these phenomena are illustrated schematically in Fig. 3. As an example it has been demonstrated that the functioning of the receptor rhodopsin in retina is controlled by non-lamellar lipids (43). A role for non-lamellar lipids in cell signaling in relation to growth processes has recently been proposed (44).

MOLECULAR SELF-ASSEMBLY IN LIPID MEMBRANES

Lipid-Protein Interactions

Due to the particular transverse and lateral structure of lipid-bilayer membranes, integral membrane proteins and receptors are subject to a variety of lipid-protein interactions that control the molecular self-assembly processes in the membrane (15,45). Firstly, the integral protein on the one hand has to adapt to the transverse bilayer amphiphilic signature (46,47) and its particular pressure profile (48). On the other hand, the lipid conformational structure and the lateral organization of the different lipid components have to adjust to the presence of the protein, as illustrated schematically in Fig. 4a. One of the important elements of the lipid-protein interactions hence involve hydrophobic matching leading to lipid sorting and lipid selectivity at the protein-lipid interface (49) as well as specific chemical and physical preference of the protein for specific lipids (50). Secondly, the lateral membrane organization as discussed above (cf. Fig. 2) will both be affected by the presence of the proteins as well as influence the lateral organization and distribution of the proteins. The particular molecular and compositional structure of the lipid annulus around the protein couples to the conformational state of the protein and may hence be involved in protein triggering events. A particular

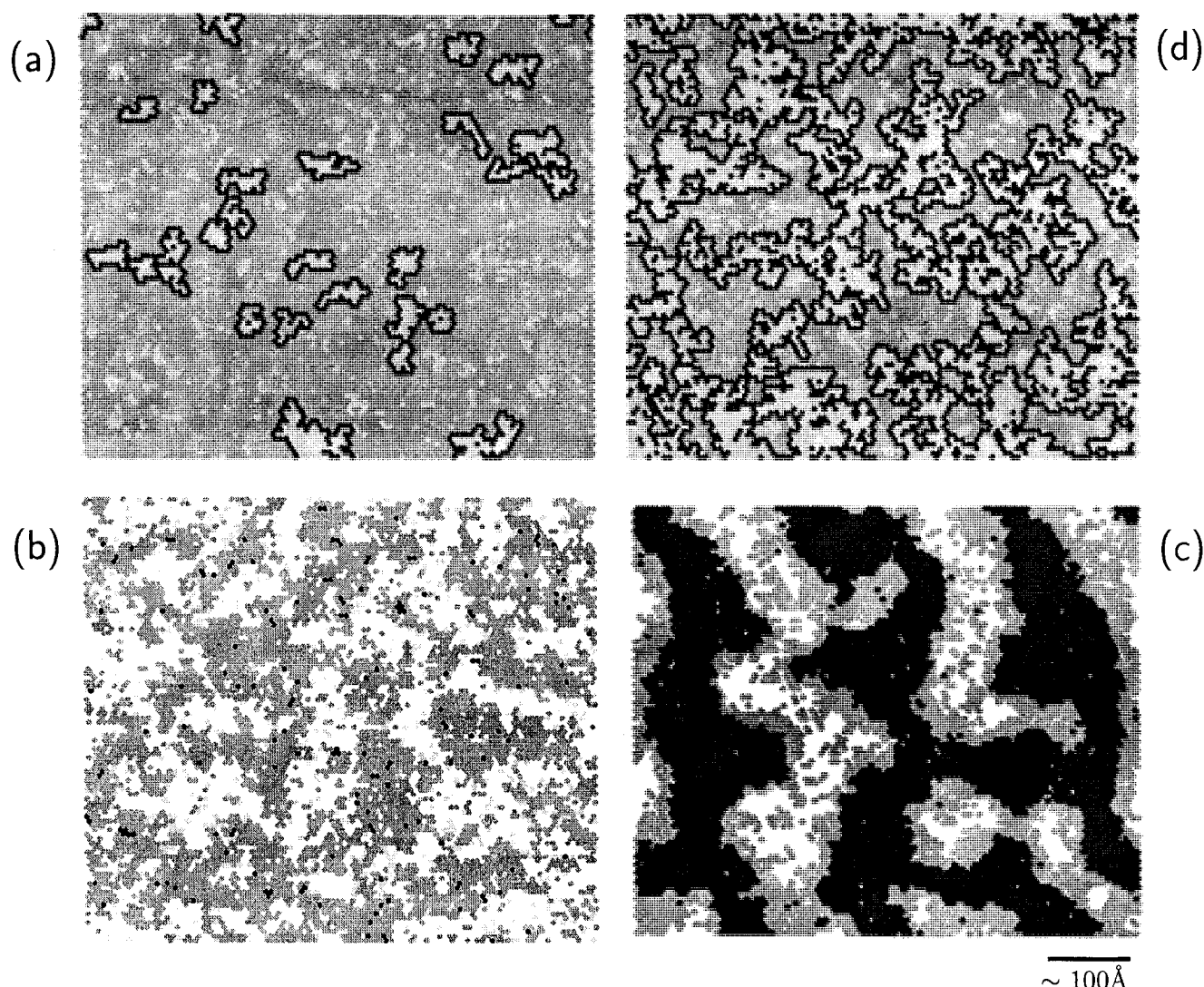


Fig. 2. Lateral structure and lipid domains in lipid bilayers as obtained from Monte Carlo calculations on model membranes. (a) density fluctuations in $DC_{16}PC$ bilayers below the main phase transition. (b) compositional fluctuations in a binary mixture of $DC_{12}PC$ and $DC_{20}PC$ in its fluid phase. (c) non-equilibrium gel-fluid lateral phase separation in a binary mixture of $DC_{12}PC$ and $DC_{18}PC$. (d) enhancement of density fluctuations in $DC_{16}PC$ bilayers in the fluid phase above the main phase transition in the presence of general anesthetics.

interesting possibility arises for lipid-mediated drug action via perturbations of the lipid annulus by the drug, or controlled access to the receptor via a special lipid environment around the receptor (51,52). An example includes the much debated mechanism of general (37,53) and local anesthetics (51).

Finally, it should be mentioned that the presence of integral proteins in the membrane influences the lipid-domain formation and hence the lateral lipid-bilayer heterogeneity (49,54,55).

Assembly of Membrane Peptides and Proteins

The direct effect of the proteins on the lipids as discussed above can promote long-range lipid-mediated forces among proteins and thereby act as a vehicle for protein associations, leading to aggregates or even two-dimensional crystals (45). These forces will be operative over distances where the particular lipid-protein profiles can overlap as shown in Fig. 4b. Under

special circumstances, that are determined by thermodynamic as well as compositional conditions, these effects can be of very long range corresponding to cases of wetting and capillary condensation (56). In those cases, protein aggregates and crystalline protein arrays can be formed as illustrated in Fig. 5 (56,57). Membrane solutes and drugs, that interact with the lipids, are expected to have a dramatic effect on this type of protein self-assembly.

CASE STUDIES

Permeability of Lipid Membranes

The permeation and transport characteristics of lipophilic and hydrophilic drugs across the lipid membrane part of biomembranes are of crucial importance for the ability of drugs to reach their target and action sites. The different types of lipids constituting the lipid membrane as well as the external

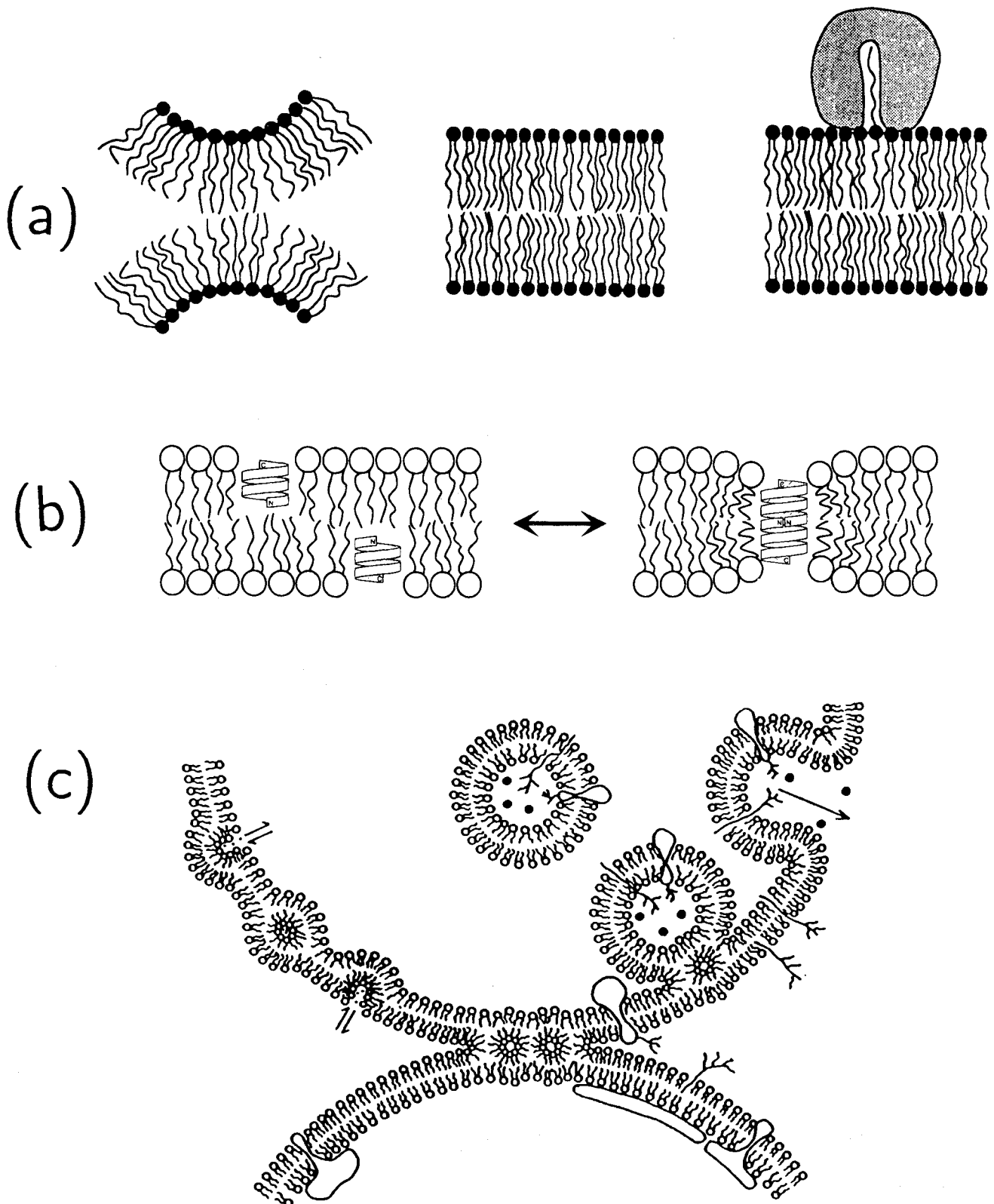


Fig. 3. Gallery of schematic illustrations of lipid-bilayer structures subject to curvature stress. (a) hypothetical situation of two relaxed lipid monolayers with intrinsic curvature corresponding to an inverse hexagonal phase (H_{II}) (left), the corresponding bilayer with built-in curvature stress (middle), and partially released curvature stress by binding to a peripheral protein by the extended chain-conformation mechanism proposed by Kinnunen (41) (right). (Courtesy by P. K. J. Kinnunen). (b) effect of a curvature stress field on the formation of the gramicidin A dimer channel. (From Ref. (42) with permission). (c) role of the formation of localized regions of non-lamellar lipid structures for a variety of membrane processes, such as cytosol, internalization, and fusion. From (4).

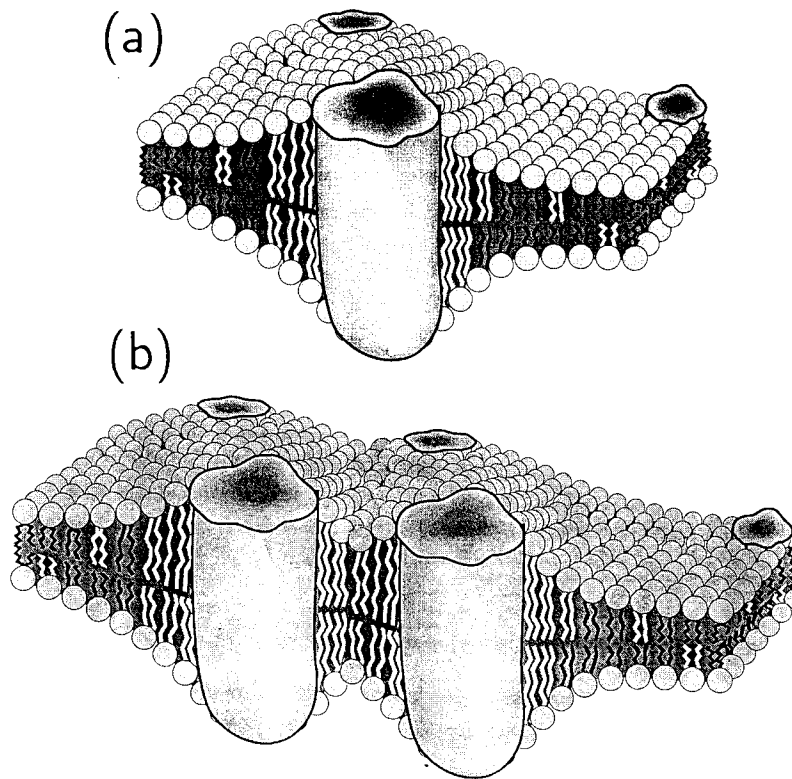


Fig. 4. (a) Schematic illustration of the hydrophobic-matching principle leading to a local profile near an integral protein embedded into a lipid bilayer. (Courtesy by M. M. Sperotto). (b) overlap of local profiles leading to lipid-mediated, indirect protein-protein attraction.

thermodynamic conditions such as temperature, pH, degree of hydration and ionic strength are all major determinants of the macroscopic phase behavior and the associated physical properties. As discussed above, lipid membranes display cooperative many-particle phenomena which can lead to the formation of lipid domains and a dynamic heterogeneous membrane structure of importance for membrane functionality. In particular, computer simulations have shown that the anomaly in the transmembrane diffusion of various molecular agents across the lipid-membrane at the main phase transition, as shown in Fig. 6 (58,59), is intimately controlled by the lipid membrane microstructure, e.g., the appearance of leaky interfacial regions

between dynamic coexisting gel and fluid domains (cf. Fig. 2a) (24,59).

The physical properties and phase behavior of the lipid-membrane part of biomembranes have been studied by various experimental techniques. Differential scanning calorimetry results of the cell wall isolated from mycobacterial cells showed two major peaks in the heat capacity at temperatures above the growth temperature of the cells (60). These results, which signaled structural phase transitions and reminiscence of phase separation phenomena, implied that a predominant amount of ordered lipids and a lipid membrane of low fluidity existed in the growing cells (60). Additionally, ESR studies using spin

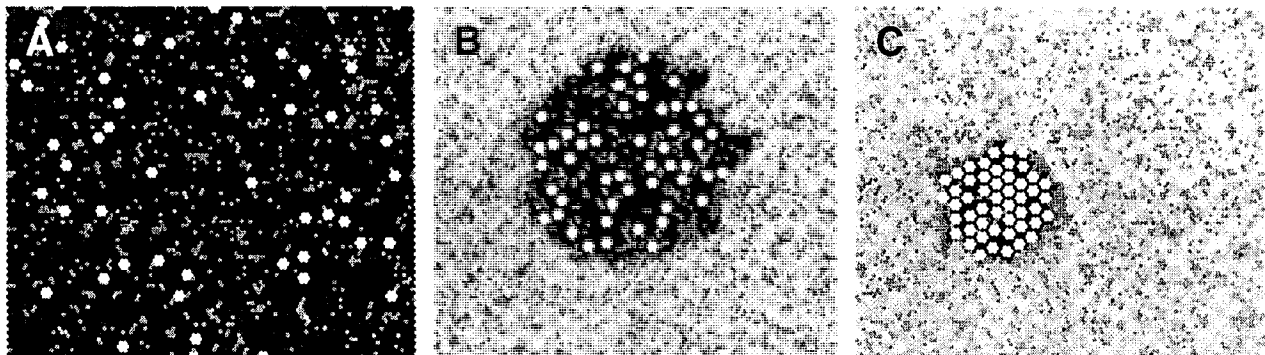


Fig. 5. Lateral distribution and organization of integral membrane proteins as obtained from Monte Carlo simulations. (A) dispersed (random) distribution. (B) aggregated proteins in a lipid domain. (C) crystalline protein array. (Courtesy by M. C. Sabra).

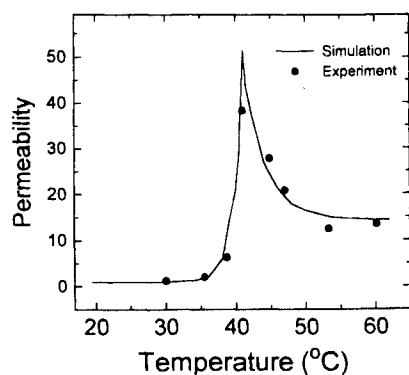


Fig. 6. Relationship between passive transmembrane relative permeability, as determined by radioactive measurements of Na^+ -ions (●)(58) and computer simulations of the lipid membrane microstructure (—) (59).

labeled fatty-acid probes, revealed an asymmetric distribution of the lipids in the cell wall membrane manifested as an internal leaflet composed of mainly ordered lipids constituting a major permeability barrier for the influx of antibacterial agents through the cell wall. These findings indicate that the physical structure of the lipid membrane and the appearance of ordered lipids in the cell wall play a significant major role for the general resistance of mycobacteria to treatment with antibiotics (60). NMR studies of a lipid membrane model of the epidermal permeability barrier and the associated phase behavior, showed that phase coexistence was common over a very broad temperature range in ternary lipid mixtures composed of ceramide, cholesterol and fatty acids, which are the lipids most often found in epidermis (61). It was observed that below 42°C a solid phase composed of ordered lipid domains with slow motion was present. The results suggested that lipid composition and lamellar organization of ordered lipid domains have a considerable effect on the diffusion characteristics and barrier properties of the epidermis (61,62). A related study of the effect of an absorption enhancer, azone, on the overall fluidity of the stratum corneum lipids demonstrated an increase in the lipid motion and a concomitant decrease in the lipid-acyl-chain order at physiological temperatures (63). A close relationship is likely to exist between the influence of the permeability enhancer on the structural and dynamical membrane behavior and the associated lowering of the lipid matrix barrier properties (63). Although a detailed understanding of the underlying molecular effects and changes exerted by permeability enhancers on the lipid membrane physical structure is still lacking, the use of permeability enhancers serve potential prospects as drug-delivery agents to promote the absorption and permeation of drug molecules to the target and action sites (64). A systematic study of the influence of a short-chain DC_{10}PC phospholipid permeability enhancer on the lipid membrane phase behavior and leakiness demonstrated a significant enhancement of the permeability over broad temperature and composition ranges (65). At temperatures corresponding to the gel-fluid phase coexistence region, a maximum in the leakiness was observed possibly due to the existence of line defects and leaky bilayer regions formed between coexisting gel and fluid phases (65). Additionally, a pronounced increase in the leakiness of the lipid membrane was induced by the short-chain lipid in the one-phase

gel and fluid regions most likely reflecting compositional fluctuations and the formation of local lipid structures (5,66).

Various membrane-incorporated molecular compounds such as cholesterol, peptides, insecticides, and drugs are able to alter the physical membrane structure and the dynamic formation of lipid domains on various length- and time scales (13,67). It has been found that low cholesterol concentrations lead to an increase in the transmembrane permeability, whereas incorporation of higher amounts of cholesterol decreases the permeability (24). These observations are in accordance with the ability of cholesterol to increase the dynamic domain formation when present in low amounts in the membrane (24). The influence of small hydrophobic compounds on the passive permeability properties can however be rather subtle. There are examples where the enhancement of the lateral heterogeneity does not necessarily lead to an increase in the permeability but rather to the opposite due to a sealing effect of the interfaces by the solute molecules which are accumulated in the interfaces (68,69,70).

In ideally behaving DC_{14}PC - DC_{16}PC mixtures a close correlation was observed between the lipid-membrane leakiness and the peak positions of the heat capacity curves, which reflects structural fluctuations and the formation of dynamic coexisting lipid domains (71,72). A related study of the more non-ideally behaving DC_{14}PC - DC_{18}PC mixture revealed a similar relationship between the membrane leakiness and the heat-capacity maxima in the vicinity of the phase lines (73). It is still an unresolved issue as to which extent the lipid mixture displays macroscopic phase separation, as dictated by the equilibrium phase diagram (74,75), or the lateral structure instead can be characterized as a non-equilibrium percolative membrane structure (20), as shown in the computer-simulated non-equilibrium snapshot of an equimolar DC_{12}PC - DC_{18}PC mixture in Fig. 2c (74). The possibility exists that local phase-separated regions are stabilized by curvature effects leading to a true equilibrium percolative structure (76). It has been demonstrated that the permeability within the phase coexistence region of a DC_{14}PC - DC_{18}PC mixture is significantly decreased as compared to the leaky membrane structure close to the phase lines (73). A deeper understanding of fundamental aspects of equilibrium and non-equilibrium lipid-membrane structure and permeability serves important drug-delivery purposes, e.g., in relation to the use of permeability enhancers to increase drug transport across biomembranes and the developments of optimized thermolabile liposomal drug-delivery systems. The use of thermolabile liposomes in combination with local hyperthermia in the body has been proposed as temperature-controlled site-specific drug-delivery systems (77). In particular, two-component temperature-sensitive liposomes characterized by phase transitions and phase separation phenomena have been tested as microcarrier systems applied to regions with localized hyperthermia (77,78).

Lipid Membrane Micro- and Macro-Structure in Relation to Liposomal Drug Delivery

The fusigenicity of liposomes is strongly influenced by the dynamical and structural properties of the lipid membrane (79). The existence of a heterogeneous lipid-bilayer structure composed of fluctuating gel and fluid domains prevailing in the temperature range of the lipid membrane main transition (cf.

Fig. 2a) leads to a strong increase in the fusion rate (80,81). Additionally, membrane curvature stress and incorporation of various non-bilayer forming agents into the lipid membrane has a dramatic effect on the fusion properties (39,82,83). In mixed lipid systems it has been shown that the proximity of a lamellar phase transition at temperatures well below the lamellar-to-hexagonal phase transition plays a major role for the ability of liposomes to undergo fusion (84). The increased fusigenicity of the mixed lipid system was understood on basis of an intimate relationship between the lipid-bilayer microstructure and the appearance of critical compositional fluctuations of lipid domains (84,85). Computer-simulation results of generic aspects of the lateral microstructure in lipid-membrane mixtures, as shown in Fig. 2b, have clearly demonstrated that the lateral membrane structure can become heavily dominated by compositional fluctuations and small-scale lipid structures in the vicinity of phase lines and close to critical points in the phase diagram (66).

Both the macroscopic phase behavior of liposomes as well as the microscopic lipid membrane organization are of importance for the *in vitro* stability and the *in vivo* fate of liposomes, e.g., the fusion and interaction of liposomes with cells and biological components of the blood stream. A significant increase in the circulation time in the blood stream has been demonstrated for liposomes containing high amounts of cholesterol (86), e.g., high cholesterol concentrations induce a so-called liquid-ordered phase characterized by tightly packed and ordered lipid acyl chains in a liquid-like bilayer matrix (87,88). Liposomes are promising microcarrier candidates in gene therapy for introducing DNA into cells (89). In particular, two-component cationic liposomes which facilitate the binding of the negatively charged DNA polymer string to the lipid-membrane surface are promising as gene delivery systems (90,91,92). The supramolecular lipid-DNA complex formed upon association of DNA with the positively charged lipid membrane surface may be of importance for the mechanisms involved in the increased transfection, e.g., liposome association and fusion with cell surfaces (89,90). Furthermore, incorporation of hexagonal-phase forming lipids, which in general increase the fusion rate of liposomes, has been found to promote transfection (93). Theoretical and experimental studies have suggested that the formation of local lipid structures with an increased local charge density in the fluid lipid membranes is an important parameter for DNA condensation at the lipid membrane surface and conversion of the lipid system into a supramolecular structure (93,94). Interestingly, computer simulations results as pictured in Fig. 2b have demonstrated that the mixing properties and the underlying phase diagram of lipid mixtures are of crucial importance for the formation of local lipid structures even far away from phase lines in the one-phase fluid regions which frequently and mistakenly are considered to be homogeneous phases (5,66). The formation of lipid domains enriched in one of the lipid species is expected to become pronounced close to both lamellar and non-lamellar phase lines and in particular in the vicinity of critical fluid-fluid demixing points in the phase diagram (5,66). A preferential interaction of the negatively charged DNA polymer string with the cationic lipids may affect the lipid mixing properties and thereby promote phase separation and phase-transition phenomena. A related effect leading to fluid-fluid phase coexistence has been reported after addition of Ca^{2+} -ions to anionic PS-PC lipid

mixtures (95). A detailed characterization of the phase diagram and phase behavior of mixed cationic liposome systems both in the presence and absence of the DNA string, might further help to optimize the material and functional properties of the composite gene-delivery lipid-based system.

The mechanical properties of lipid membranes are strongly influenced by the membrane microstructure, e.g., the existence of a dynamic heterogeneous membrane structure prevailing in the vicinity of phase transitions has a pronounced influence on the lipid-membrane bending rigidity (25). Small amounts of membrane incorporated compounds such as cholesterol and short-chain phospholipids have similar effects on the dynamic domain formation and the bending rigidity (96). In particular, such results are of interest in relation to the potential use of transfersomes as drug carrier system where the conformational deformability and softness of the lipid membrane is of importance for the ability of intact transfersomes to penetrate the skin barrier spontaneously (27,97).

Enzyme and Protein Function Controlled by Lipid-Membrane Physical Structure

Numerous experimental and theoretical investigations have demonstrated that the physical properties and phase behavior of the lipid membrane play an important role for the functioning of lipid-membrane-associated and integrated enzymes and proteins (13). A particular striking example concerns the activity of phospholipase A_2 which is a small water-soluble enzyme that is ubiquitously present in extra- and intracellular compartments of human tissues (98). Phospholipase A_2 catalysis involves adsorption of the enzyme to the lipid-membrane interface followed by hydrolysis of the phospholipids at the catalytic site of the enzyme (98). Both experimental and simulation results as shown in Fig. 7 have demonstrated that the activity of phospholipase A_2 depends strongly on the physical state and microstructure of the lipid-membrane substrate (33,34). In particular, a pronounced temperature and acyl-chain-length dependent phospholipase A_2 activity is observed in the transition region implying a strong correlation between the enzymatic activity and the lipid-membrane microstructure, e.g., the formation of a heterogeneous lateral membrane structure composed of dynamic lipid domains and a network of interfacial regions as illustrated in Fig. 2a. In addition, both the lateral surface stress and the incorporation of non-bilayer forming lipids into the membrane matrix can give rise to an increased enzymatic activity (35,99).

Important drug-delivery aspects are related to an improved understanding of the *in vivo* destabilization and interaction of biological components and lipid-membrane degrading enzymes with polymer-grafted liposomes. Surface modified PEG liposomes have come into widespread use not only as microcarrier drug-delivery systems with improved therapeutic profiles (100,101), but also as simple model systems to study surface properties and phase behavior of polymer-grafted lipid membranes (102,103,104,105). Both experimental and theoretical investigations of the phase structure of polymer-grafted liposomes have clarified that a bilayer-to-micellar transition takes place at a certain lipopolymer threshold concentration depending upon the length of the PEG polymer (102,103,105). Furthermore, a change in the configuration of the polymer chains from a so-called mushroom to a brush structure takes

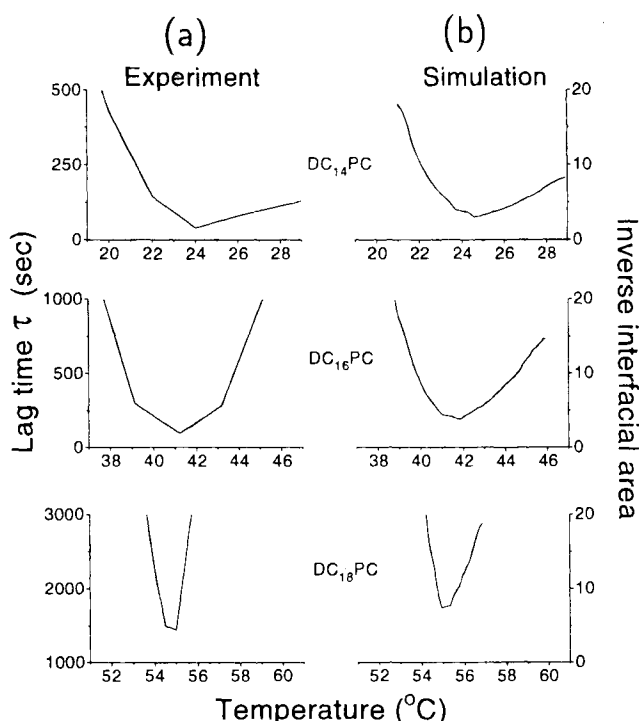


Fig. 7. Relationship between phospholipase A₂ activity and lipid membrane microstructure. (a) phospholipase A₂ lag time curves. The lag time is inversely related to the activity of the enzymatic hydrolysis of unilamellar DC₁₄PC, DC₁₆PC, and DC₁₈PC liposomes. (b) Monte Carlo computer simulation of the temperature dependence of the inverse interfacial area of the heterogeneous lipid membrane structure (cf. Fig. 1a). Results are shown for liposomes composed of DC₁₄PC, DC₁₆PC, and DC₁₈PC lipids (33,34).

place at small concentrations of the lipopolymer in the membrane (104,106). The lipopolymers are generally assumed to establish a steric barrier that prevents rapid recognition and clearance of the liposomes in the blood stream by the reticuloendothelial system (107,108). However, the detailed molecular mechanisms underlying the the extravascular degradation of polymer-grafted liposomes are poorly understood (109,110). A systematic study of the activity of phospholipase A₂ towards polymer-coated liposomes revealed a remarkably lipopolymer concentration-dependent increase in phospholipase A₂ activity over broad temperature ranges in the transition region (110). Such results suggest that the enhanced enzymatic activity of phospholipase A₂ towards polymer-grafted liposomes is of importance for the extravascular degradation of polymer-grafted liposomes, e.g., in pathological tissue due to a combined effect of an elevated concentration of active phospholipase A₂ (111,112), an increased catalytic activity towards lipopolymer-containing liposomes (110), and an accumulation of polymer-grafted liposomes in such tissue (100).

The functioning of several membrane-associated enzymes and proteins are strongly influenced by the physical properties of the lipid membrane. It has been found that protein kinase C requires negatively charged PS lipids in order to bind to the membrane surface and to become active at interfacial lipid domains containing an increased local concentration of diacylglycerol (29). Similarly, local lipid structures and the formation of lipid-membrane domains enriched in certain lipids have been

reported to be of importance for the activity of the acetylcholine receptor channel (113).

Incorporation of non-bilayer forming lipids into the lipid membrane, which modulate the propensity for forming hexagonal phases, play a central role for lipid-membrane stability and protein functioning (39). A particular clear example is related to the ability of the antibacterial gramicidin A monomer peptide to form dimer membrane-spanning ion-conducting channels (114) as shown in Fig. 3. The dimerization and channel formation is strongly influenced by non-lamellar forming lysolipids which release the curvature stress at the membrane-peptide interface induced by the hydrophobic mismatch between the thickness of the membrane and the ion-conducting dimer channel (114,115). A deeper understanding of the interplay between lipid-membrane structure and the activity of membrane-spanning proteins and peptides might furthermore be of particular interest in relation to the functioning of integral proteins as active transmembrane transporters of certain drugs, e.g., in relation to the effect of lipid composition on the multidrug resistant MDR1 P-glycoprotein activity (116) and the potential use of the di-tripeptide carrier as active transporter of peptide prodrugs (117).

Lipid Membranes as Target Sites for Drugs. Molecular Mechanisms of Drug Action?

The molecular mechanisms involved in the pharmacological effects exerted by a variety of drugs upon binding to proteins and lipid membranes is a question of fundamental importance in molecular pharmacology (118). It is well-known that a large number of drugs with different chemical structures and pharmacological effects are able to bind to lipid membranes and alter the membrane physical properties (119,120,121). Examples include general and local anesthetics, non-steroidal anti-inflammatory agents and calcium channel blocking drugs (122,123,124). In particular, the influence of local and general anesthetics on the lipid membrane structure and dynamics has been extensively studied (51,53,121). General anesthetics include chemical structures as diverse as small halogenated agents, alcohols with different chain length, and bulky steroid compounds, obviously reflecting an apparent lack of structure-activity relationships. In addition, the only known antagonist of anesthesia is hydrostatic pressure which is a bare physicochemical quantity (53). In spite of numerous experimental and theoretical studies in the field of anesthesia, there is still no clear conception as to whether the molecular mechanisms mainly involve specific receptor-based interactions with lipid membrane proteins or non-specific effects on the lipid-membrane physical properties, which in turn are of importance for the optimal functioning of integral and peripheral proteins (51,121). A recently proposed hypothesis relates the molecular action of anesthetics to a change in the lateral pressure profile of the lipid membrane and a subsequent effect on the conformational flexibility and function of membrane-spanning proteins (125). NMR studies have suggested that the interaction and binding to the interfacial lipid membrane-water interface, which is followed by a disruption of the hydration shell, is involved in the target and action sites for amphiphilic agents (126). In accordance with this, isothermal titration calorimetry has revealed a positive enthalpy of binding alcohol to the membrane, corresponding to a release of structured water molecules (127).

Furthermore, a close correlation between the ability of anesthetic and non-anesthetic isomers to bind at certain positions at the interfacial water-membrane region has been demonstrated (128,129). Heat capacity measurements of the influence of general anesthetics on the lipid membrane thermodynamics showed a dramatic influence on the phase behavior (130). Interestingly, this effect was partly reversed by application of hydrostatic pressure (130). Computer-simulation results as shown in Fig. 2d have suggested that the ability of anesthetics to increase the dynamic membrane heterogeneity may involve a preferential interaction with certain lipid structures (37). An increased affinity of drugs to differentiated regions of the heterogeneous membrane structure is of particular interest in relation to a preferential interaction at the lipid-protein interface and a concomitant perturbation of the local lipid-protein environment (cf. Figs. 4a) of crucial importance for the optimal functioning of membrane attached proteins (45,52,115). Additionally, it has been suggested that a preferential association and orientation of certain flexible opioid peptide drugs upon binding to the lipid-membrane interface may facilitate drug-receptor interactions (131).

SUMMARY

We have in this mini-review revisited the conventional fluid-mosaic model of the fluid lipid-bilayer component of biological membranes and pointed out that contrary to common belief, lipid-bilayer membranes are highly structured fluids, both transversely and laterally. In particular we have discussed the structural ordering in the nanometer range and we have pointed to observations that clearly show that this type of organization is important for membrane function and hence for drug action as well as for the use of liposomes for drug-delivery purposes. A greater understanding and appreciation of the subtleties of lipid-membrane structure and how this structure can be manipulated in the time- and length-scale regimes relevant for drug action holds a promise for novel developments in pharmaceutical sciences.

ACKNOWLEDGMENTS

This work was supported by the Danish Natural Science Research Council and the Danish Technical Research Council. The authors are affiliated with the Danish Centre for Drug Design and Transport supported by the Danish Medical Research Council. Members of the MEMPHYS group at the Technical University of Denmark are thanked for discussions on various aspects of the work reviewed in the present paper. OGM is an Associate Fellow of the Canadian Institute for Advanced Research.

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