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REVIEW ARTICLE

Conformation and mode of organization of amphiphilic membrane components: a conformational analysis

Robert BRASSEUR and Jean-Marie RUYSSCHAERT
Laboratoire Chimie-Physique des Macromolécules aux Interfaces, Université Libre de Bruxelles, CP 206/2, Boulevard du Triomphe, 1050 Bruxelles, Belgium

INTRODUCTION

Phospholipids and proteins constitute the major components of biological membranes. They are known to occur in various proportions ranging from approx. 25% lipid in the mitochondrial inner membranes to 80% lipid in myelin. They retain, however, the basic structural features and presumably play the same functional role in all membranes. Consideration of biological membranes at the molecular level requires a detailed knowledge of the preferred conformations of phospholipids and of the mode of insertion of amphiphilic membrane components (drugs, proteins) into the lipid matrix. Phospholipids dispersed in water often spontaneously form bilayer membranes, although other non-lamellar phases are also found depending on composition and temperature (Luzzati, 1968; Cullis & de Kruijff, 1979). X-ray diffraction, neutron diffraction (Hauser *et al.*, 1981) combined with the use of deuterated material and i.r. spectroscopy (Fringeli & Günthard, 1969) have provided information about the position and orientation of molecules in lipid bilayers. This molecular approach is a prerequisite in the understanding of the functions and organization of the biological membrane. Since these experimental approaches have been described extensively in a previous review (Hauser *et al.*, 1981) the present article will focus on conformational analysis as another way to gain insight into the structure of membrane components. However, as often as possible, the theoretical predictions will be compared with the experimental data. For considerations of computing time or because of the limits of theoretical methods, investigators have often limited their analysis to distinct regions of the isolated molecule (polar head group, acyl chain, entire molecule). We will, however, describe the few attempts that have been made to give a molecular description of the assembly mode of phospholipids in monolayers or bilayers. Finally, examples will be given illustrating the correlation between the mode of insertion of a drug or a membrane component into the lipid matrix and the modifications of its pharmacological or biological functions. To facilitate a comparison between the results obtained, we will use for the phospholipid the atom numbering and the notation for torsional angles proposed by Sundaralingam (Sundaralingam, 1972) (Fig. 1).

The large number of theoretical models for a lipid bilayer is symptomatic of the complexity of the physical situation. Since an exact theoretical solution to this problem is not yet possible, two lines of approximate solutions have been essentially considered. One approach is to simplify the actual physical situation into one that can be solved exactly or by controlled approximations. It is the most rigorous and has the most physical meaning. Such procedures (quantum-mechanical studies) have been essentially used to calculate the structure of isolated lipids or of fragments of lipids (hydrophilic moiety, acyl chain). Another approach (semi-empirical) tries to solve the general model by resorting to approximations that may, in part, lose physical meaning. It has been chosen in order to give a molecular description of the lipid structure in organized systems (bilayer, micelle, inverted micelle). The choice of this type of model results from necessities of reproducing the experimental data but should not be envisaged as a permanent alternative to other theoretical approaches. As will be shown further, this analysis reproduces the experimental results with accuracy and it is only because

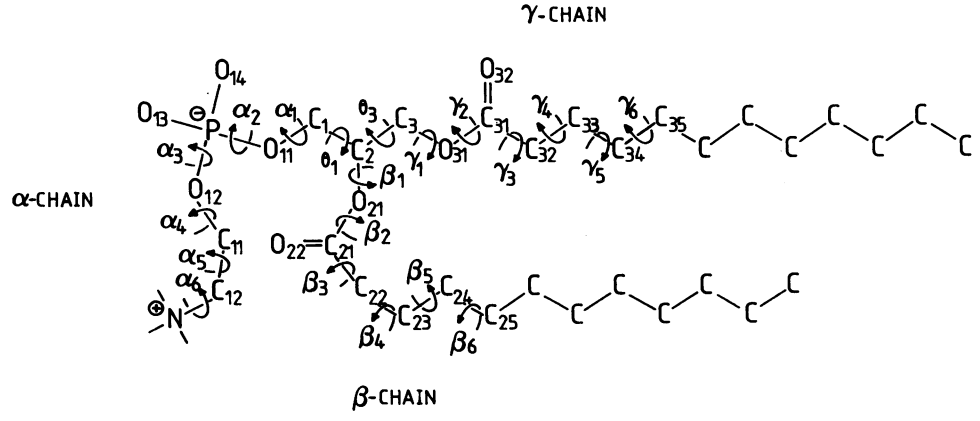

Fig. 1. Atom numbering and notation for torsion angles according to Sundaralingam (1972), illustrated for phosphatidylcholine

of this accuracy that the basis of specific experimental data can be understood.

LIPID STRUCTURE

Isolated lipid molecule

Polar head group. Sundaralingam (1972) predicted from the X-ray structural features of glycerolphosphorylcholine, its cadmium complex, glycerolphosphorylethanolamine, fatty acid, diacylglycerol and triacylglycerol, six possible models for the phospholipid conformation. In each of them, the glycerol oxygen atoms of the acyl ester functions are in the *gauche* conformation. The choline and the phosphate residues adopt various conformations which could result from modifications in the crystal packing forces. The quantum-mechanical studies of Pullman *et al.* (1975) demonstrated that no difference should be expected between ethanolamine phosphate and choline phosphate with respect to the conformation about the α_5 rotational angle. An excellent agreement was observed for the value of the α_4 rotational angle in the conformational analysis and the X-ray crystallographic study of 1,2-dilauroyl-DL-phosphatidylethanolamine (Hitchcock *et al.*, 1974). For the other rotational angles of the polar group, the discrepancies between the two approaches underline the importance of the changes in the orientation of the polar head group during the packing process. It should also be kept in mind that the structure of the isolated polar head group could be different from that of the same group in the entire molecule; it is a major weakness of this kind of approach limited to one region of the lipid molecule. The optimization of the polar head-polar head group interaction results from the orientation of the NH_3^+ and PO_4^- residues. The packing indeed optimizes the intermolecular electrostatic interaction between cationic and anionic groups. Pullman & Berthod (1974) underline the evidence that the polar heads of the phospholipids show an intrinsic preference toward highly folded structures with strong intramolecular hydrogen bonds. Their existence in the open form in the crystals and possibly in water must be attributed to the effect of the intermolecular forces. Scott (1975) and Kreissler *et al.* (1983) take the water molecules explicitly into account. The first represents the water medium by a surface occupied at the polar heads interface. The second represents the hydrophilic medium by one or more water molecules around the solute polar head group. They calculate the hydration sites characterized by geometric parameters and the hydration energy by an *ab initio* method. The ethanolamine phosphate head group offers two hydration centres, the ammonium and the phosphate moieties. The intermolecular interactions are evaluated with the Lenard–Jones potential and a Coulomb potential for the electrostatic term with $\epsilon = 20$. One water molecule interacts with the phosphate and three water molecules with the ammonium moiety. Recently (Scott, 1984, 1985) the potential and the orientational order of water has been calculated between two head groups (choline phosphate, ethanolamine phosphate, serine) interfaces. In the Monte Carlo procedure, 172 water molecules were located between two lipid bilayers separated by 2.45 nm (24.5 Å). The choline phosphate headgroup in an ordered array induces a dipolar ordering in the nearby water layer. When choline phosphate heads are completely disordered the induced dipolar order is minimal although the steric presence of the polar heads influence the location of the water layers.

The hydrocarbon chain. Salem (1962) analysed in detail the long-range intermolecular forces responsible for the stability of lipoproteins. Special attention is drawn to the dispersion forces and to the conditions under which these forces are locally additive; accurate values of the dispersion energy of interaction between saturated hydrocarbon chains at short distances (a few Å apart) are calculated by adding all the bond–bond interactions. A general expression is given for the dispersion energy between two parallel and opposed chains built out of identical units, and numerical values are given for the case of closely packed hydrocarbon chains. The total attraction energy is extremely sensitive to the intermolecular distance. The role of this distance specificity in interactions involving unsaturated fatty acid chains and its contribution to the stability of lipoproteins is examined. At large distance [> 1–2 nm (10–20 Å)] only electrostatic forces will be really significant and the London–Van der Waals dispersion energy is maximal in monolayer films for a distance equal to 0.48 nm (4.8 Å) between two saturated hydrocarbon chains.

A statistical mechanical treatment of the fluidity of lipid hydrocarbon chains in phospholipid bilayers was performed by Marsh (1974). With an effective energy separation of 3140 J/mol between *gauche* and *trans* conformations, it is found possible to account both for the chain dependence of the entropy and for enthalpy change at the lipid crystalline transition of saturated lecithins. The model proposed by Scott (1974) exhibits, under certain circumstances, two phase transitions, one corresponding to the positional disordering of entire lipid molecules, and the other corresponding to orientational disordering in the hydrocarbon chains.

Bell *et al.* (1974) calculate theoretically two different structural properties of aliphatic bimolecular layers: the melting entropy of saturated chains and the variation in thickness of the bilayer during melting. Jacobs *et al.* (1975) and Scott (1975) use a statistical method to simulate the melting phase transition of phospholipids. The model of Jacobs *et al.* (1975) includes interactions between head groups, between hydrocarbon chains and within the chains. The head groups are modelled as hard disks which are constrained to lie on a two-dimensional surface. The chain statistic problem is treated in an approximate manner using the excluded volume repulsion and longer range attractions. The experimental data (transition temperature and melting enthalpy) obtained for 1,2-dipalmitoylphosphatidylcholine were used as references in the evaluation of the theoretical parameters for lipids with different chain lengths. This model contains approximate treatments of hard-core repulsions and weak attractive forces between molecules. In a more recent paper, Scott (1977) uses a Monte Carlo approach in order to describe the hydrocarbon region of lipid bilayers. The first carbon atom in each chain is confined to a plane of specified size, and the remainder of the chains lie below the plane, simulating a monolayer or half bilayer. The remaining carbon atoms in each chain are generated by multiple applications of the rotation operator. Each Monte Carlo experiment is begun with the chains uniformly spread throughout the available volume and in an all-*trans* state. A *gauche* rotation is

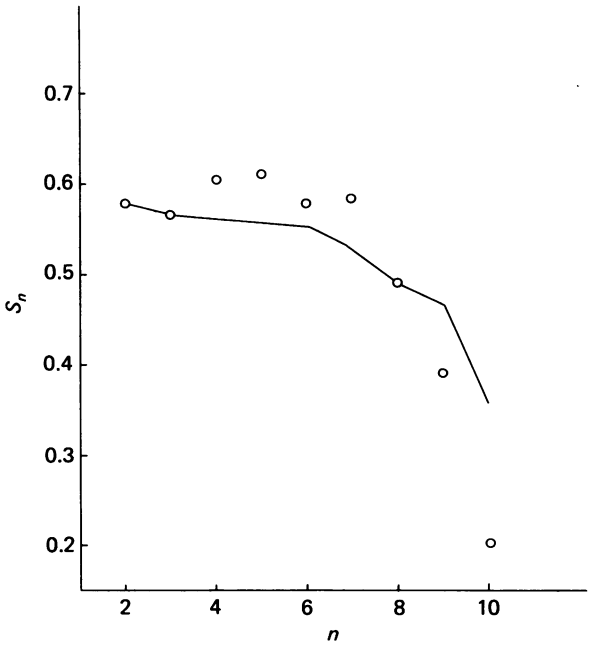

Fig. 2. Calculated order parameter (s_n) versus the atom position (n) compared with ^{2}H-n.m.r. data (from Scott, 1977)

performed at the randomly chosen bond. In addition, the chain is rotated through a random angle about the long axis.

Fig. 2 indicates the good correlation between the calculated order parameters (S_n) and the ^{2}H magnetic resonance data. Caille *et al.* (1980) developed a model for the first-order gel–fluid phase transition in lipid mono- and bilayers. The lipid chains are assumed to lie on a triangular lattice and to interact via anisotropic Van der Waals forces. A two-state Ising-like model is developed and shown to give a good qualitative description of the main phase transition of both mono- and bilayers and of statistical fluctuations about this transition. The phase diagrams with their corresponding phase separation regions, the heat of transition, and in some cases the correlations are calculated for different systems. Dill & Flory (1980), using a lattice model for a liquid, investigated the packing of short-chain molecules in interphases such as a bilayer membrane. The constant density at the interface imposes intermolecular constraints on the configurations of the flexible chains. The statistical theory predicts a diffuse distribution of chain ends near the bilayer midplane; a good correlation was observed between the calculated disorder profile and the experimental data. Gruen (1980) presented a statistical mechanical model of a bilayer of dipalmitoyl-3-*sn*-phosphatidylcholine molecules above their phase transition. The model shows a good agreement between several experimental results and a theoretical analysis of the order parameter profile across the bilayer. Melardi & Schlitter (1981*a*,*b*) presented a statistical mechanical treatment of fatty acyl chain order in phospholipid bilayers. The theoretical approach is compared with experimental data (^{2}H-n.m.r., neutron scattering, calorimetry) obtained for a dipalmitoyl-3-*sn*-phosphatidylcholine bilayer. The main criticism of this comparison is that, as for the polar head group, the structure of the isolated acyl chain can only be roughly compared with the structure of this chain in the entire lipid molecule. Finally, very recently, Kirk *et al.* (1984) proposed a thermodynamic model of the lamellar to inverse hexagonal phase transition of lipid. A free energy per lipid molecule is calculated for each phase as the sum of four lattice-specific terms: a local elastic term and global terms involving the packing of hydrocarbon chains, Debye-shielded electrostatic interactions and hydration effects. The local curvature free energy depends on two parameters, the curvature elasticity and the equilibrium radius of curvature. These parameters must be temperature-dependent. An equilibrium average molecular area would then be generated by minimizing the average local molecular free energy together with the long-range hydration and electrostatic effects that depend simply on average molecular area.

Entire molecule. McAlister *et al* (1973) calculated the preferred conformation of the diacyl phosphatidylcholines taking into account the Lenard–Jones potential function. The inclination of the two hydrocarbon chains makes it possible to optimize their mutual interactions essential in the intermolecular packing of phospholipids in membranes. The α chain can exhibit several conformations as a result of different energetically likely conformations for the phosphodiester and choline moieties. Pullman & Saran (1975) using a PCILO method carried out a procedure in three steps: (i) structure of the central glycerol moiety including the first few torsional angles of the acyl chains, (ii) structure of the extremity of the polar head group, and (iii) arrangement of α, β and γ chains. Their main conclusions are: (i) there are two preferred folded conformations of the α chains, and (ii) the two acyl chains can adopt a limited number of structures.

Comparison between the values of torsional angles and the values corresponding to the crystallographic conformations reveal a difference for the structure at the extremity of the polar head group. It could be due to the formation in the crystal of an intermolecular hydrogen bond between the terminal cationic head of one molecule and a negative oxygen of the phosphate group of the neighbouring one. Brosio *et al.* (1977) proposed two structures for the P–N dipole. In a first case, a unique topological situation is allowed with the P–N dipole lying parallel to the lattice plane. In the second case, different situations including that with the P–N dipole lying orthogonal to the plane are allowed. Brasseur *et al.* (1981) followed a strategy currently used to study the conformations of polypeptides (Ralston & De Coen, 1974). The method was modified to incorporate variations in the dielectric constant and the energy of transfer from one environment to another at the simulated lipid–water interface. To limit the number of possible degrees of structural freedom, bond lengths and bond angles were assumed to be constant. The total conformation energy is calculated as the sum of the following terms.

(1) *The London–Van der Waals energy of interaction between all pairs of non-mutually-bonded atoms.* Buckingham's pairwise atom–atom interaction functions have been used:

$$E^{VdW} = \sum_{ij}[A_{ij}\exp(-B_{ij}r_{ij}) - C_{ij}r_{ij}^{-6}] \qquad (1)$$

where $ij = 1,2,\ldots$ are non-bonded atoms, r_{ij} their

distances from each other, and A_{ij}, B_{ij} and C_{ij} are coefficients assigned to atom pairs. The values of these coefficients have been reported by Liquori and co-workers (Liquori *et al.*, 1968; Giglio *et al.*, 1968). Like other quantum-mechanical results, these values emerge in part as the solution of the Schrödinger equation and in part as heuristic variables. They have been applied with success to conformational analysis of molecular crystals, proteins, polypeptides and lipids. In order to compensate for the decrease of the function E^{VdW} at small r_{ij}, we have imposed an arbitrary cut-off value of:

$$E^{VdW} = 418.4 \text{ kJ/mol (100 kcal/mol) at } r_{ij} < 0.1 \text{ nm (1 Å)}$$

(2) *The generalized Keesom–Van der Waals interaction or electrostatic interaction between atomic point charges:*

$$E^{cb} = 332\left(\sum_{ij} \frac{e_i e_j}{r_{ij}\epsilon_{ij}}\right) \quad (2)$$

where e_i and e_j are expressed in electron charge units and r_{ij} in Å. ϵ_{ij} is the dielectric constant. The values of atomic point charge are similar to those used for polypeptides (Hopfinger, 1973).

(3) *The potential energy of rotation of torsional angles.* This rotation around the C–C or C–O bonds was calculated by the equation:

$$E^{Tor} = \frac{U_{ij}}{2} \cdot (1 + \cos\phi_{ij}) \quad (3)$$

where U_{ij} corresponds to the energy barrier in the eclipsed conformation during the rotation of the angle, and ϕ_{ij} is the torsional angle. U_{ij} is equal to 11.7 kJ/mol (2.8 kcal/mol) for the C–C bond and 7.5 kJ/mol (1.8 kcal/mol) for the C–O bond.

(4) *The transfer energy of each part of the molecule.* The values of the transfer energies used ($E^{+}_{transfer}$) are similar to those determined experimentally by numerous authors, as summarized elsewhere (Tanford, 1973).

Table 1 (Bellemare & Fragata, 1980; Fragata & Bellemare, 1980) summarized the values of dielectric constant (ϵ) in model membranes determined experimentally and by theoretical estimation. To simulate the membrane interface, Brasseur assumed a dielectric constant equal to 3 above the interface, while at the atom most deeply immersed in the aqueous phase a plane was lined where the dielectric constant was assumed to be 30. Between these two planes, the dielectric constant was assumed to increase linearly along the z-axis perpendicular to the interface. The molecule is finally oriented with the line joining the hydrophilic and hydrophobic centres perpendicular to the interface (Brasseur *et al.*, 1983*a*) (Fig. 3). The hydrophilic centre ($\vec{C}^{phi}_{tr}$) is defined by the following equation:

$$\vec{C}^{phi}_{tr} = \sum_{i-1}^{n} (E^{+}_{transfer_i} \vec{r}_i) / \sum_{i-1}^{n} E^{+}_{transfer_i}$$

in which $\vec{r}_i$ are the co-ordinates of the i atom. The hydrophobic centre located in the hydrocarbon domain ($\vec{C}^{pho}_{tr}$) is defined by the same equation, except that the

Table 1. Micropolarity data (ϵ) on model membranes determined experimentally and by theoretical estimation

Abbreviations used: ANS, 1-anilino-8-naphthalenesulphonic acid; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; ϵ, dielectric constant; *NN'*-DOC, *NN'*-di(octadecyl)oxacarbocyanine; PC, phosphatidylcholine; TOC, α-tocopherol. (From Bellemare & Fragata, 1980.)

Model membrane	Site of determination	Method	ϵ	Reference
Single-layered vesicles (PC)	Vesicle wall	Dielectric dispersion	10–30	Schwan *et al.* (1970)
Lipid bilayers	Polar head: near bulk water	Theoretical estimation	40	Gillespie (1970)
Single-layered vesicles (PC) of radius:	Vesicle wall of 3.5 nm (35 Å)	Dielectric dispersion		Redwood *et al.* (1972)
15 nm (150 Å)			13.9	
14 nm (140 Å)			15.1	
12.5 nm (125 Å)			17.3	
Multilayers (PC)	Lipid–water interface	Theoretical estimation	19	Colbow & Jones (1975)
Multilayers (PC)	Polar head interface: probably near bulk water	Fluorescence polarity probe: ANS	32	Colbow & Chong (1975)
Multilayers (PC)	Polar head interface: near hydrocarbon core	Fluorescence polarity probe: *NN'*-DOC	25	Colbow & Chong (1975)
Single-layered vesicles (PC,TOC)	Hydrogen belt–hydrocarbon core interface	Chemical reaction probe: DPPH-TOC	24.6 ± 1.0	Bellemare & Fragata (1980)

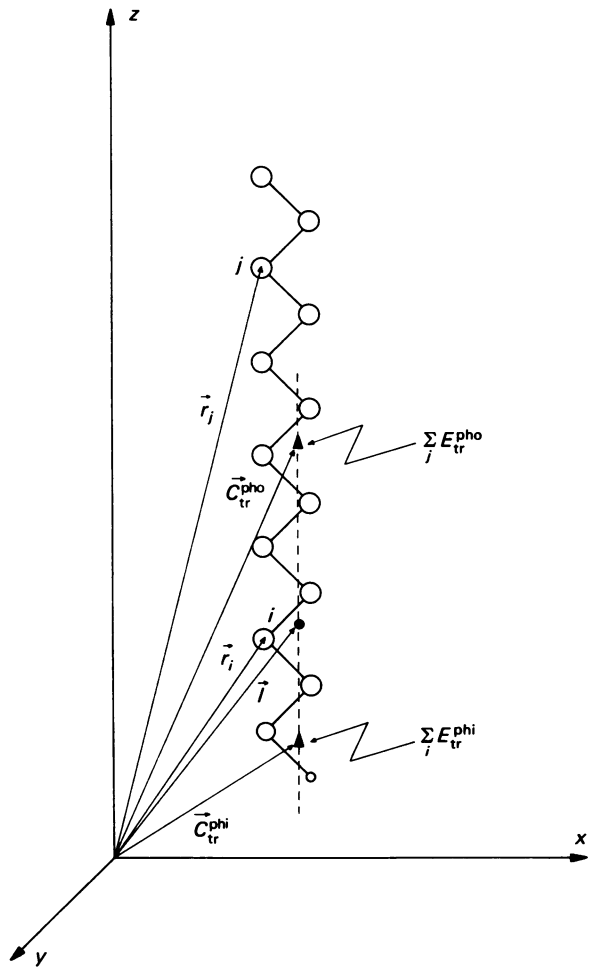

Fig. 3. Definition of the co-ordinates ($\vec{r}_i$) of the hydrophobic centre ($\vec{C}_{tr}^{pho}$), the hydrophilic centre ($\vec{C}_{tr}^{phi}$) and the interface $\vec{I}$

negative transfer energies are taken into account. The interface position ($\vec{I}$) is defined by the equation:

$$\frac{\sum_{i=1}^{n} E^{+}_{\text{transfer}_i}}{\vec{C}_{tr}^{phi} - \vec{I}} = \frac{\sum_{j=1}^{m} E^{-}_{\text{transfer}_j}}{\vec{C}_{tr}^{pho} - \vec{I}}$$

Lipids assembled in monolayers or bilayers

We have discussed up to this point only the structure of the isolated lipid molecule. Although the approaches used have a high degree of sophistication, they will not satisfy the biochemist since lipids, in membranes, are organized in bilayers. However, the assemblage of isolated lipid molecules implies the development of procedures which mimic the lipid organization. Kreissler & Bothorel (1978) have proposed a model making it possible to mimic the melting transition. It shows that an increase of the interchain distance between lipids during melting does not notably modify the conformational energy of the glycerol moiety. However the number of stable conformations decreases as the distance increases and eventually, the glycerol moiety becomes rigid. Kreissler *et al.* (1983) present a theoretical conformational analysis of a system composed of seven interacting dipalmitoylphosphatidylethanolamine molecules. The combined use of classical semi-empirical methods for the polar headgroup region with mechanical statistical calculations for the aliphatic chains permits the evaluation of the free energy for a phospholipid molecule. The free energy variation as a function of the mean intermolecular interchain distances gives information about the main lipid bilayer phase transition. It appears necessary to take into account the hydration of the polar head group or the anisotropy of the interface. The most fascinating work is probably the theoretical prediction of the molecular organization in micelles and vesicles described by Dill and coworkers (Dill & Flory, 1980; Dill, 1982; Dill *et al.*, 1984). The configurations of the hydrocarbon chain in micelles are severely constrained by the space-filling requirements of the chain segments and by the micellar geometry. Dill & Flory (1981) developed a statistical method taking into account the constraints in micelles. The chain disorder near the outside of the hydrophobic core may approach that of a liquid and crowding of the chains near the core centre imposes a degree of order approaching that in a crystal. Also, they envisaged the effects of curvatures on the chain configurations in monolayers and bilayers. The disorder gradient in the inner and outer half-bilayers of small vesicles should be substantially different. The data indicate that the net disorder is greater in the vesicle than in the planar bilayer. Recently Dill *et al.* (1984) proposed a configuration of a 38-chain spherical dicanoate micelle according to the interface theory (see Fig. 3 of that paper). This method gives an elegant representation of the degree of chain disorder, the distribution of termini and the extent to which hydrocarbon segments, including chain ends, are exposed to the solvent at the core interface. The structures and properties of this micellar aggregate are thus found to be the consequences of Langmuir's principle of differential solubility, according to which hydrocarbons are sequestered into a core devoid of water surrounded by the polar head, the principle that steric forces determine essentially the structures of condensed phase, and the statistical mechanical principle that degrees of freedom of equal energy (for the intramolecular chain configuration) are of equal likelihood.

A similar approach has been proposed by Israelachvili *et al.* (1977). Brasseur *et al.* (1981) developed a procedure of assemblage which can be summarized as follows.

For monolayer formation:

(*a*) The position of lipid B is modified along the *x* axis (Fig. 4*a*). Each distance change is equal to 0.05 nm (0.5 Å) (the phosphate atom was used to define the distance separating two molecules). For each separating distance a rotation angle of 30° is imposed on lipid B around its own *z* axis and around lipid A (Fig. 4*b*). Among 14400 possible orientations only the structure of energy minimum was considered.

(*b*) Lipid A position is fixed and lipid B is allowed to move along the *z* axis perpendicular to the lipid water interface (Fig. 4*c*). Again, only the structure of energy minimum is considered.

(*c*) Lipid B has the possibility to change its orientation around the *z* axis as compared with lipid A (Fig. 4*c*). This procedure allows us to define finally the probable packing of the two lipid molecules. Addition of a third molecule to the two preceding supposes a similar

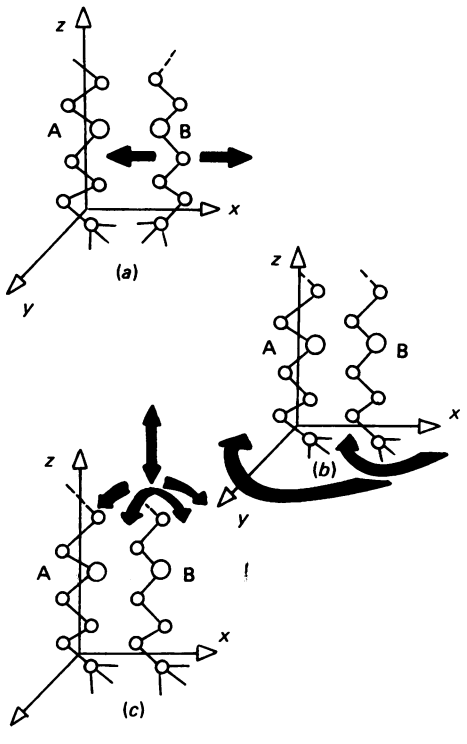

Fig. 4. Schematic presentation of the packing procedure of lipid molecules assembled in monolayers

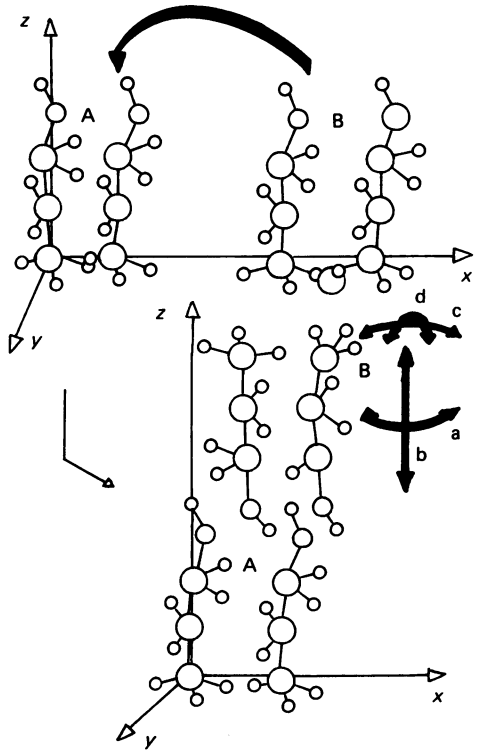

Fig. 5. Schematic presentation of the packing procedure of lipid molecules assembled in bilayers

approach. The packing of the first two molecules is maintained and one studies the orientation of the third molecule around them.

For bilayer formation:

(*a*) Monolayer B position is modified along the *z* axis. Again each separating distance is equal to 0.1 nm (1 Å) (Fig. 5*b*). A rotation angle of 30° is imposed on monolayer B around the *z* axis (Fig. 5*a*). Monolayer A is fixed.

(*b*) Monolayer B has the possibility to change its orientation around the *z* axis (Figs. 5*d* and 5*c*). Monolayer A is fixed.

In each case, only the bilayer structure of energy minimum is retained. For dipalmitoylphosphatidylcholine, the most probable conformation is characterized by the close proximity of the phosphate residue associated with the hydrophilic moiety of one lipid and the choline residue associated with the adjacent lipid. The electrostatic interaction between the two residues stabilizes the lipid structure. From the structure of the lipid in the monolayer, the organization in the lipid bilayer was theoretically evaluated in an attempt to compare it with recent experimental data. Indeed, neutron diffraction combined with the use of selectively deuterated lipids can provide detailed information about the molecular structure (Buldt *et al.*, 1978). This approach has been recently applied to bilayer membranes of dipalmitoylphosphatidylcholine deuterated at 12 different positions in the hydrocarbon chain and polar head group. The distances obtained experimentally and theoretically between the centre of the bilayer and each deuterated atom were in excellent ageement (Table 2). The starting point for the theoretical evaluation was the conformation of the individual molecules calculated according to Brasseur *et al.* (1981). The isolated molecules were assembled as described (Brasseur *et al.*, 1981). This theoretical approach is performed without introducing any parameter associated with the crystal structure. This method has also been applied successfully to predict other modes of organization of lipid aggregates such as micelles or hexagonal H_{II} types of configuration (Brasseur *et al.*, 1983*c*, 1984*b*). Although it is still too premature today to define any dimensional parameter asociated with these calculated structures, in view of the limited number of associated molecules used in the calculations, the present results demonstrate the feasibility of the prediction of aggregate structures from molecular conformational analysis.

STRUCTURE OF AMPHIPHILIC MOLECULES INSERTED INTO A LIPID MATRIX

Drugs and sterols

Scott & Cherng (1978) developed a Monte Carlo technique to study the effects of protein, cholesterol, bilayer curvature and mobility on the chain order parameters of a lipid layer. Simulations of protein and cholesterol effects are accomplished by insertion of a rigid stationary cylinder into the lipid matrix. The effect of cholesterol on the order and packing of hydrocarbon region depends on the penetration of the sterol into the hydrocarbon region. The effect of an increased cholesterol concentration would be to make the chains more rigid above the penetration depth, and still allow for fluidity

Table 2. Summary of the mean carbon atom positions of dipalmitoyl phosphatidylcholine in the bilayer

Method	Distance from the centre of the bilayer (nm)										
	C_γ	$C\beta$	C^α	GC-3	C 2 (2)	C-2 (1)	C-4	C-5	C-9	C-14	C-15
Neutron diffraction studies (Buldt *et al.*, 1978)	2.51±0.06	2.48±0.07	2.45±0.07	2.31±0.1	2.0±0.1	1.81±0.1	1.62±0.06	1.5±0.06	1.01±0.1	0.41±0.06	0.29±0.06
Theoretical conformational analysis (Brasseur *et al.*, 1981)	2.52	2.5	2.4	2.22	2.02	1.73	1.56	1.47	1.0	0.47	0.35

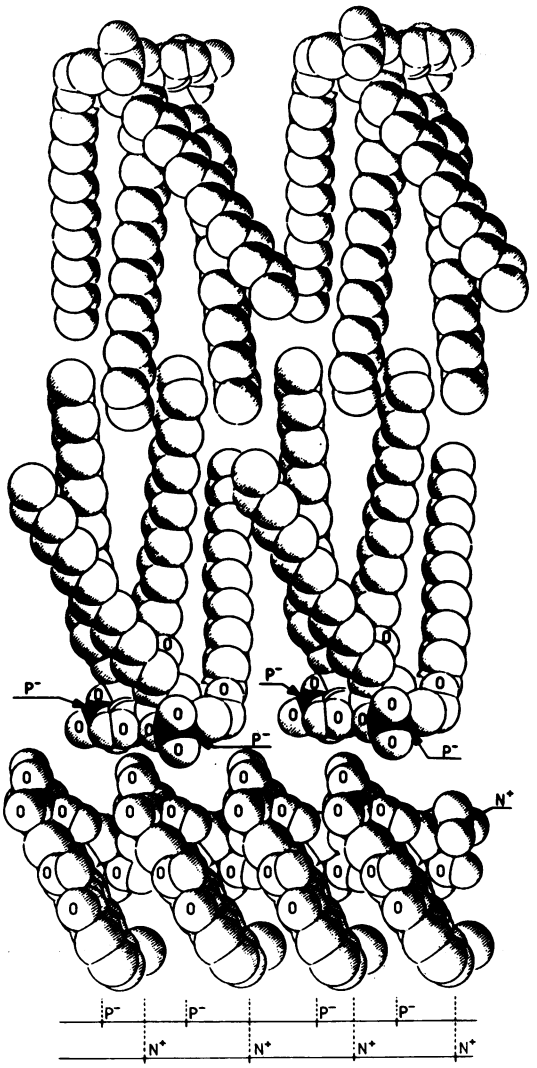

Fig. 6. Computer picture of two cardiolipin molecules assembled with four adriamycin molecules

Arrows indicate the position of the lipid phosphate groups (P^-) and of the adriamycin amine groups (N^+). The mean distance between charged phosphate groups in the same and in adjacent cardiolipin molecules is equal to the mean distance between adriamycin charged amino groups. The plane–plane interactions between adriamycin molecules stabilize the cardiolipin cluster formations responsible for the adriamycin cardiotoxicity.

in the regions below this depth. More recently O'Leary (1983) proposed a simple theoretical model describing the effects of molecules inserted in biomembranes. A random mixing assumption is used in conjunction with a molecular model for the phase transition in order to describe the major effects of cholesterol on biomembranes. The model accounts for the cholesterol-induced membrane condensation above the gel-to-liquid-crystalline phase transition. Recent data illustrate how conformational analysis makes it possible to correlate the mode of insertion of a drug into a lipid matrix with its pharmacological activity.

Aminoglycoside antibiotics induce a lysosomal phospholipidosis in kidney proximal tubules after conventional therapy in animals and man (De Broe *et al.*, 1984). It was demonstrated that these drugs bind to

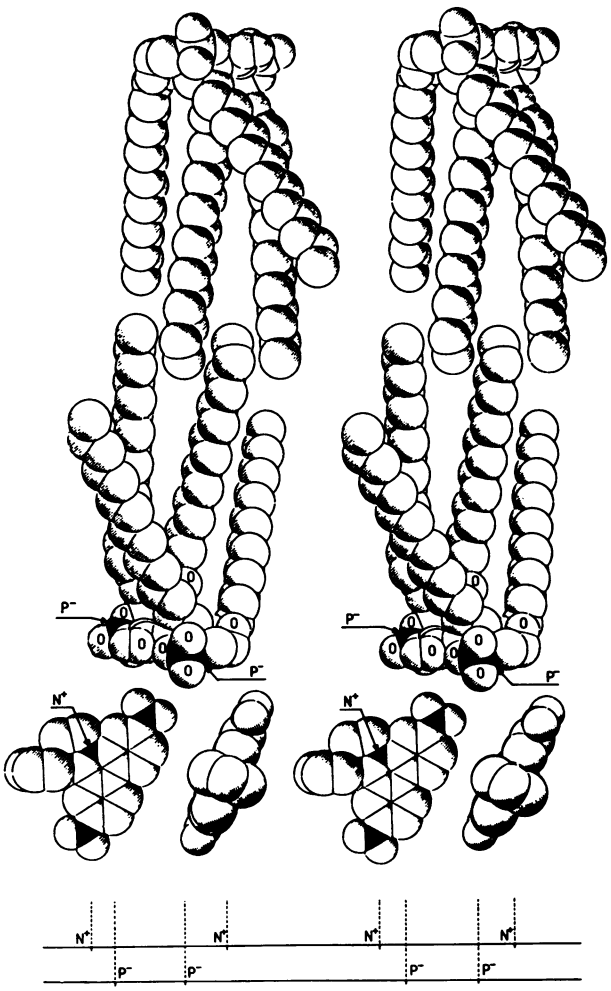

Fig. 7. Computer picture of two cardiolipin molecules assembled with four ethidium bromide molecules

P^- and N^+ have the same meaning as in Fig. 6. The plane–plane interactions are made impossible by the drug structure and the complex obtained exist as a monomer in the membrane. The repulsion between the cardiolipin molecules is illustrated by a greater distance between the cardiolipin molecules.

negatively charged phospholipid bilayers at acid pH and inhibit the activity of lysosomal acid phospholipases *in vitro* and *in vivo*. A combined biochemical and conformational study (Brasseur *et al.*, 1984*a*) shows major differences between 6-aminoglycosides in current clinical use with respect to the stability of the complexes they form with phosphatidylinositol, their inhibitory potency towards the activity of lysosomal phospholipases and their current toxicity. This study was extended to streptomycin derivatives (Brasseur *et al.*, 1985*a*). All streptomycin derivatives extend parallel with the fatty acid chains and across the hydrophilic–hydrophobic interface. Gentamicin, in contrast, lies parallel with this interface and above the plane of the phospho groups. The mode of insertion of the aminoglycosides into the bilayer appears as crucial as the binding to negatively charged lipids.

Phorbol esters and diacylglycerols activate protein kinase C but specific structural parameters appear to be required for the enzyme activation. From the conformational analysis of potent and non-potent diacylglycerols and phorbol esters, specific regions crucial for potency in activating the enzyme were identified. The data suggest that the conformational approach could be used to design rationally specific inhibitors preventing the effects of tumour promoters and to predict the structure of potential tumour promoters (Brasseur *et al.*, 1985*d*). Adriamycin is one of the most active antitumour agents against leukaemia and solid tumours. However, its cardiotoxicity notably limits the total dose that may be given. Since cardiolipin has been presented as a target responsible for the cardiotoxicity (Duarte-Karim *et al.*, 1976; Goormaghtigh *et al.*, 1982) an attempt was made to elucidate the geometry of the complex formed and the molecular reasons responsible for its high stability. More insight into the spatial organization was obtained from the conformational analysis. A linear crystal-like organization of the complex appears (Goormaghtigh & Ruysschaert, 1984). The adriamycin molecules lie parallel, tilted at 36° with respect to the normal to the bilayer plane (Fig. 6). The mean distance between the positive charges located in the adriamycin molecule in this linear organization is exactly identical with that obtained between the negative charges of cardiolipin in the close packed structure. Since the data strongly suggest a correlation between the mitochondrial toxicity of the drug and its capability to induce cardiolipin clusters formation, it was tempting to design new structures avoiding the lipid cluster formation but preserving the interaction with DNA. To illustrate this approach, ethidium bromide, which has for cardiolipin an identical affinity with that of adriamycin, was chosen. The computer picture given in Fig. 7 demonstrates clearly that for ethidium bromide, the plane–plane interactions between adjacent drug molecules are made impossible by the ethidium bromide tridimensional structure and the complex obtained with cardiolipin exists as a monomer in the membrane. Steric repulsions occur when two complexed monomers are in close proximity. The repulsion is illustrated by a greater distance between the cardiolipin molecules, and the space between the two complexed cardiolipin molecules will be filled by other phospholipid molecules in the real membrane. The most fascinating result is probably that ethidium bromide shows no mitochondrial toxicity (Huart *et al.*, 1984); moreover, the two drugs have very similar affinity for DNA. This conformational analysis has also made it possible to give a molecular picture of the mode of insertion of antimycotics (Brasseur *et al.*, 1984*a*), propranolol (Brasseur *et al.*, 1985*c*) and alcohols (Brasseur *et al.*, 1985*b*) into the lipid matrix and to understand the relationship between the orientation and the pharmacological mode of action.

Ionophores

Ionophores are compounds that facilitate the transfer of ions across lipid bilayers. They are capable of forming with ions lipid-soluble complexes (carriers) which, by virtue of their apolar nature, can cross membranes by diffusion. Some of them form channels (pore formers) in the membrane, facilitating ion passage from one lipid–water interface to another.

Carriers. This ion-transfer process across the lipid membrane supposes the existence of two conformations

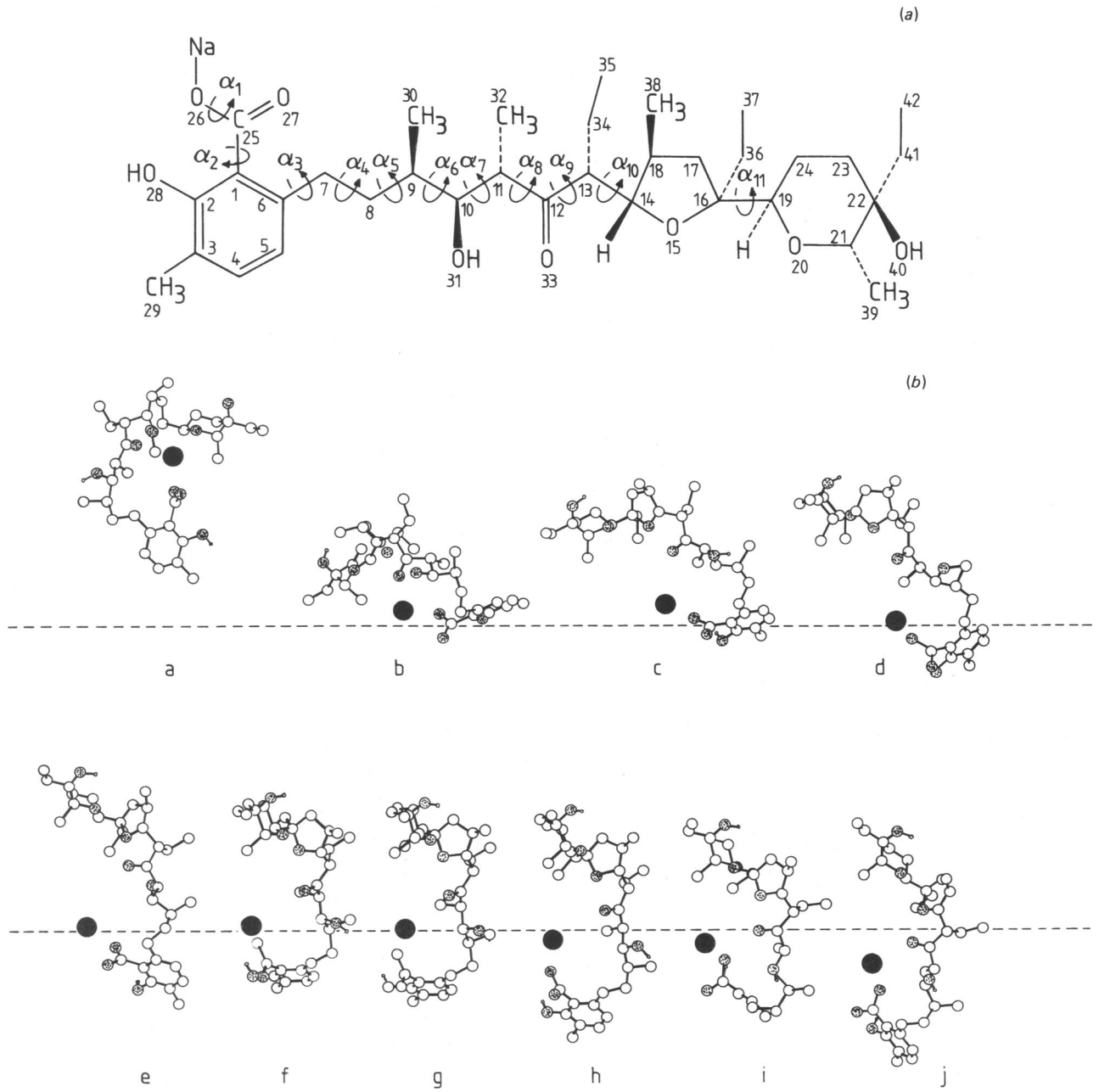

Fig. 8. (*a*) Structural formula of lasalocid–Na^+ complex with numbering of atoms and torsional angles, and (*b*) sequence of decomplexation process of lasalocid–Na^+ complex at a simulated lipid-water interface

The complex is seen to be transformed from a cyclic form in an apolar environment (a) to be quasi-linear conformation in the high dielectric medium (j) when it is pushed through the interface. Black, dotted and open circles refer to Na, O and C atoms respectively. The broken line represents the membrane interface discontinuity.

of the ionophore: a lipophilic conformation capable of conveying the ion across the hydrocarbon region of the cell membrane and an interfacial conformer responsible for the ion complexation/decomplexation process at the lipid–water interface. It is however very difficult to obtain experimental information on these transient conformations of the ionophore–ion complex formed at a membrane–water interface. In this environment, the dielectric constant will vary greatly (ranging from approx. 2.5 to 40) over a distance smaller than the ionophore size so that a part of the molecule may stay in a pure hydrophobic environment whereas another part may reach a high-polarity environment. Since its X-ray analysis (Johnson *et al.*, 1970), the conformation of lasalocid A, which complexes alkalis, alkaline earths and amines, has been extensively investigated (Fig. 8*a*). Because of the presence of a relatively long and flexible chain bearing polar groups, the ionophore can adopt a wide variety of conformations in solvents of different polarities. Application of the conformational analysis (Brasseur *et al.*, 1984*c*) for a medium of dielectric constant equal to 3 gives a structure very similar to the bulk-phase configuration derived from X-ray analysis. The sodium ion is complexed by five oxygen atoms in the crystal structure determination (O-15, O-20, O-31, O-33 and O-40) and by four oxygen atoms in our structure

(O-15, O-20, O-31 and O-33). Only the position of the O-40 differs in the two configurations. This difference is probably due to the crystallization conditions. Indeed, one molecule of methanol is associated with one molecule of the lasalocid A–Na^+ complex and the O-40 acts as hydrogen-bond donor to the methanol molecule. To mimic the immersion of the membrane-bound ionophore into the aqueous phase, the interface simulated by the dielectric constant change was imposed at several positions of the ionophore and for each case the procedure of minimization and reorientation at the interface was applied. Each position corresponds to a different degree of immersion of the ionophore into the aqueous phase. It is obvious from Fig. 8 that during the passage through the interface the Na^+ ion leaves its cryptic position (Fig. 8*b*, a). The ionophore adopts progressively a much more extended conformation more favourable to Na^+ complexation or decomplexation at the interface (Fig. 8*b*, j). Painter *et al.* (1982) have proposed that in high-polarity solvents, the uncomplexed anionic ionophore assumes an acyclic conformation and as the solvent polarity decreases, the conformation shifts toward a cyclic structure. Cyclization proceeds by rotation about three C–C hinge bonds (α_5, α_8, α_9). Our scenario suggests that the transconformation of the Na^+/X537A complex from a cyclic form to an acyclic form is obtained essentially by the rotation about five C–C hinge bonds (α_4, α_5, α_7, α_8, α_9). The approach used by Painter *et al.* (1982) considers neither the orientation of the molecule at the membrane–water interface nor the Na^+–lasalocid complex. Conformational change is the result, in this case, of a change of the solvent dielectric constant but the authors do not consider the possibility of a structure modification mediated by the membrane–water interface. The same procedure was applied to other carriers (Brasseur *et al.*, 1982, 1983*b*; Brasseur & Deleers, 1984) (A23187, ionomycin) and demonstrated also this kind of transconformation at the lipid–water interface. The approach described makes it possible to predict the ionophoretic properties of drugs before their synthesis and offers a unique way to identify transient conformations of ionophores crossing lipid membranes.

Channel formers. Gramicidin A is a linear pentadecapeptide which in a dimer conformation forms ion-selective channels in membranes. The mode of insertion of gramicidin in a lipid bilayer has been extensively studied and four classes of models have been proposed: the *N*-terminal to *N*-terminal $\beta^{6.3}$ helical dimer (Urry *et al.*, 1983*a*), the *C*-terminal to *C*-terminal helical dimer (Bradley *et al.*, 1978) and parallel (Arseniev *et al.*, 1985) and anti-parallel double helices. The *N*–*N*-terminal $\beta^{6.3}$ helical dimer was originally proposed by Urry *et al.* (1983*b*) as the channel conformation and is presently largely accepted on the basis of ^{13}-C-n.m.r., i.r. and c.d. studies. However, there are two aspects of the molecule which are difficult to understand with such an orientation. Firstly, intuitively a location of the *C*-terminal part at the membrane-water interface seems unlikely in view of the concentration of the four hydrophobic tryptophan residues at that end of the molecule. Secondly, the profound effect of gramicidin on lipid polymorphism e.g. a bilayer stabilization on lysophosphatidylcholine systems (Killian *et al.*, 1983) and hexagonal H_{II} phase induction in phosphatidylcholine and phosphatidylethanolamine system (Van Echteld *et al.*, 1982) can be understood assuming a conical shape of the molecule in which the bulky tryptophans are located towards the centre of the bilayer. With these apparent discrepancies in mind, we (R. Brasseur, V. Cabiaux, J. A. Killian, B. de Kruijff & J. M. Ruysschaert, unpublished work) calculated the most probable orientation of gramicidin at a lysopalmitoylphosphatidylcholine–water interface. The assembly procedure has been applied to gramicidin A and lysopalmitoylphosphatidylcholine. If gramicidin A was assumed to orientate its tryptophans towards the hydrophobic phase, four lysopalmitoylphosphatidylcholine molecules were calculated to surround one gramicidin molecule. In this complex, the total calculated area occupied by the hydrophobic moieties [2.5 nm^2 (250 $Å^2$)] and the hydrophilic moieties [2.4 nm^2 (240 $Å^2$)] are almost identical; the dynamical shape associated with such a structure is cylindrical which is compatible with a bilayer organization, in agreement with the experimental data. For the gramicidin structure oriented with the tryptophans pointing towards the aqueous phase, six lysophosphatidylcholine molecules are required to surround one gramicidin A. Since the area occupied per hydrophilic moiety is larger [1.37 nm^2 (137 $Å^2$)] than for the hydrophobic moiety [0.47 nm^2 (47 $Å^2$)] a dynamical shape similar to that of lysophosphatidylcholine is obtained, making a lamellar organization highly unlikely. Even if the conformational analysis procedure is still crude and does not take into account possible conformational changes of the gramicidin–lysophosphatidylcholine aggregate resulting from changes in the gramicidin conformation induced by the interaction with the lipid, it is a first tentative attempt to predict the mode of insertion of a polypeptide into the lipid matrix.

CONCLUSIONS

We hope to have shown that conformational analysis makes it possible to reach a molecular description of the assemblage mode of amphiphilic membrane components like phospholipids and to predict the mode of insertion of amphiphilic molecules into a lipid matrix. To establish the validity of the prediction, a comparison with the experimental data is a prerequisite. This comparison has been made in a few cases but should be extended to a large number of compounds. It should also be kept in mind that further refinements are required to resolve some limiting factors of the conformational analysis procedure, especially the fact that it does not take into account possible conformation changes resulting from molecular interactions. Also, extensive efforts should be made to extend the theoretical analysis in order to describe the mode of penetration of proteins into a lipid matrix. For proteins containing a large number of amino acids, this analysis would be preceded by a prediction of the hydrophobic region (Eisenberg, 1984; Kyte & Doolittle, 1982).

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