Theoretical analysis of membrane molecular organization

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A new semiempirical conformational analysis procedure has been developed to give a molecular description of the mode of organization of amphiphilic molecules. The feasibility of this approach for phospholipids, major components of biological membranes is illustrated. The method can be extended to any amphiphilic molecule to give a molecular description of its mode of insertion into a lipid matrix. The implementation of the procedure using a CDC Cyber 170 coupled with an Olivetti M40 are outlined.

Keywords: phospholipids, lipid membranes, conformational analysis, molecular membrane organization

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Phospholipids are major constituents of biological membranes. Their amphiphilic structure is responsible for their tendency to aggregate in organized structures in aqueous media. Recent results have shown their crucial role in the functioning of membranes. Lipids are essential for the maintenance of membrane biological activity, such as enzyme activity, demonstrated by cytochrome C oxidase which requires cardiolipin in its environment to be active. Inactivation of mitochondrial enzymes due to a specific drug–lipid interaction[1] has been described for cardiolipin and adriamycin. The purpose of this paper is to provide a molecular description of this kind of interaction, and also the mode of assembly of lipid molecules. The theoretical analysis of molecular membrane organization (TAMMO) is proposed to consist of three steps (see Figure 1) namely: orientation of the isolated molecule at the lipid–water interface, monolayer formation and bilayer formation.

THEORETICAL BASIS OF THE CONFORMATIONAL ANALYSIS PROCEDURE

Energy fields

The conformation of an isolated molecule and its orientation at the lipid–water interface has been established previously[2,3]. The total conformational energy, which is the sum of the contributions resulting from Van der Waals interactions, the torsional potential, the electrostatic interactions and the transfer energy is calculated for a large number of conformations orientations and assemblage modes.

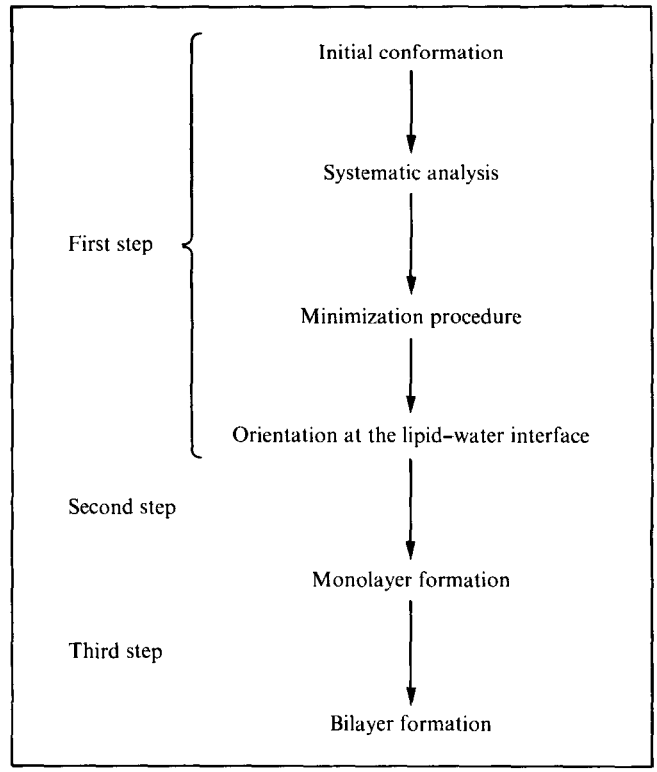

Figure 1. Schematic representation of the theoretical analysis of model membrane organization (TAMMO)

Conformation and orientation of isolated molecules

Six changes of 60° each were first imposed to each of n torsional angles, yielding 6^n conformers. The conformational energy was calculated for each of these conformers. The most probable configurations were taken as those yielding the lowest internal energy i.e. those with a statistical-weight of at least 1%. The values used for valence angles and bond lengths were used in conformational analysis[4]. After systematic analysis, conformations selected for their lowest internal energy were submitted to a Simplex minimization procedure[5]. To simulate the lipid–water interface, a dielectric constant

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ε_{ij} equal to 3 above the interface was adopted while the atom at the bottom of the molecular configuration was fixed in a plane where the dielectric constant is 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 gravity centres perpendicular to the interface. The hydrophilic gravity centre ($\vec{C}_w$) is defined by the following equation:

$$\vec{C}_w = \frac{\sum_i E_{tri}^{phi} \vec{r}_i}{\sum_i E_{tri}^{pho}}$$

in which $\vec{r}_i$ are the coordinates of the i atom and E_{tr}^{phi} the hydrophilic transfer energy of the atom i[6]. The hydrophobic centre located in the hydrocarbon domain ($\vec{C}_{hc}$) is defined by the same equation, except that the negative transfer energies are taken into account. The interface position ($\vec{I}$) is defined by the equation:

$$\frac{\sum E_{tr}^{phi}}{\vec{C}_w - \vec{I}} = \frac{\sum E_{tr}^{pho}}{\vec{C}_{hc} - \vec{I}}$$

Monolayer formation

The procedure of assembly of the monolayer is as follows:

a) The position of molecule B is adjusted along the x axis (see Figure 2(a)). The position of molecule A is fixed. Each distance change is equal to 0.5 Å. For each separating distance, a rotation angle of 30° is imposed on molecule B around its own z axis and around molecule A (see Figure 2(b)). Among all possible orientations only the structure of energy minimum is considered.

b) Molecule A has its position fixed and molecule B is allowed to move along the z axis perpendicular to the lipid–water interface (see Figure 2(c)). Again, only the structure of energy minimum is considered.

c) Molecule B is able to change its orientation around the z axis compared to molecule A (see Figure 2(c)). This procedure allows the final definition of the probable packing of the two molecules. The addition of a third molecule B (to the two preceding ones) supposes a similar approach. The packing of the first two molecules is maintained and the orientation of the third molecule around them is studied.

Each of the structures obtained for the isolated lipid molecule was assembled in a monolayer. (This was limited to an assembly of 9 to 13 molecules).

Formation of bilayers

The procedure used is the following:

a) Monolayer B position is modified along the z axis. Again each separating distance is equal to 1 Å (see Figure 3(b)). A rotation angle of 30° is imposed to monolayer B around z axis (see Figure 3(a)). Monolayer A is fixed.

b) Monolayer B has the possibility to change its orientation around the z axis (see Figure 3(d) and 3(c)). Monolayer A is fixed. In each case, only the energy minimum bilayer structure is retained.

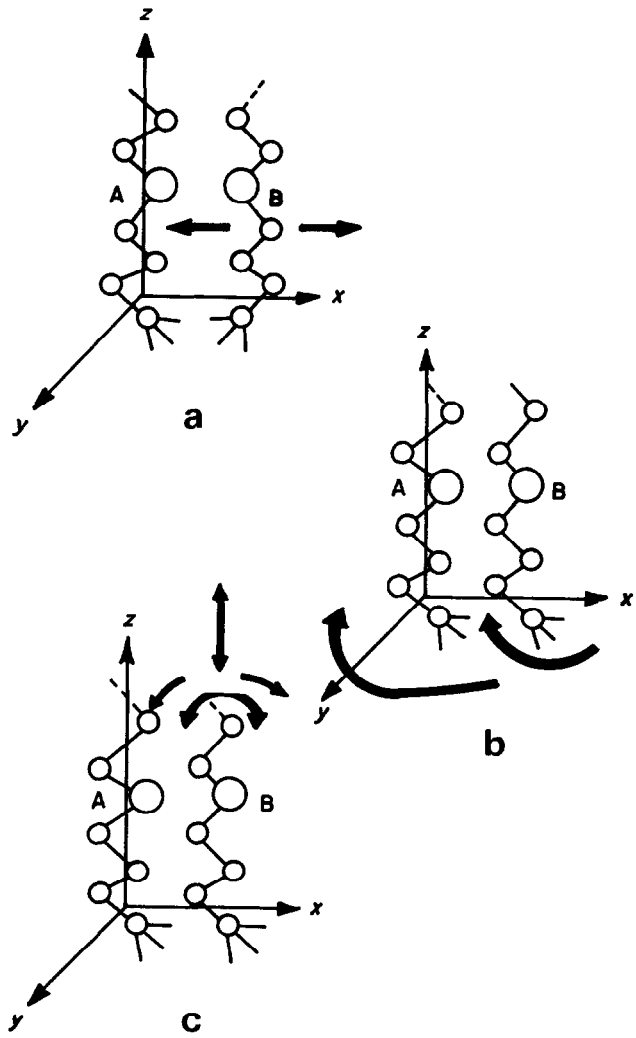

Figure 2. Schematic representation of the packing procedure of lipid molecules assembled in monolayers

IMPLEMENTATION OF THE PROCEDURE INTO THE COMPUTER SYSTEM

Computer system

Calculations were performed on a CDC Cyber 170 computer coupled to a Calcomp 1051 drawing table (Computing Centre of the Brussels Free University) and also an Olivetti M40 through a RS232C interface. All programs for the CDC Cyber 170 were written in FORTRAN IV, whereas the programs for the RS232C interface and the Olivetti M40 were written in BASIC.

Procedure organization for isolated molecules

A first interactive program (see Figure 4), named CHANO, makes it possible to define all the geometrical and energetical parameters associated with one molecule (file Ageom). The systematic analysis was performed by the CHAN2B program and the energy–probability map by the CHAN2C program. The molecule with the highest energy probability was submitted to the Simplex minimization procedure (the CHAN2A program)[5]. The orientation of the molecule at the lipid–water interface is produced by the CHAN2D program. The Aori file containing all the parameters of the molecule A (co-

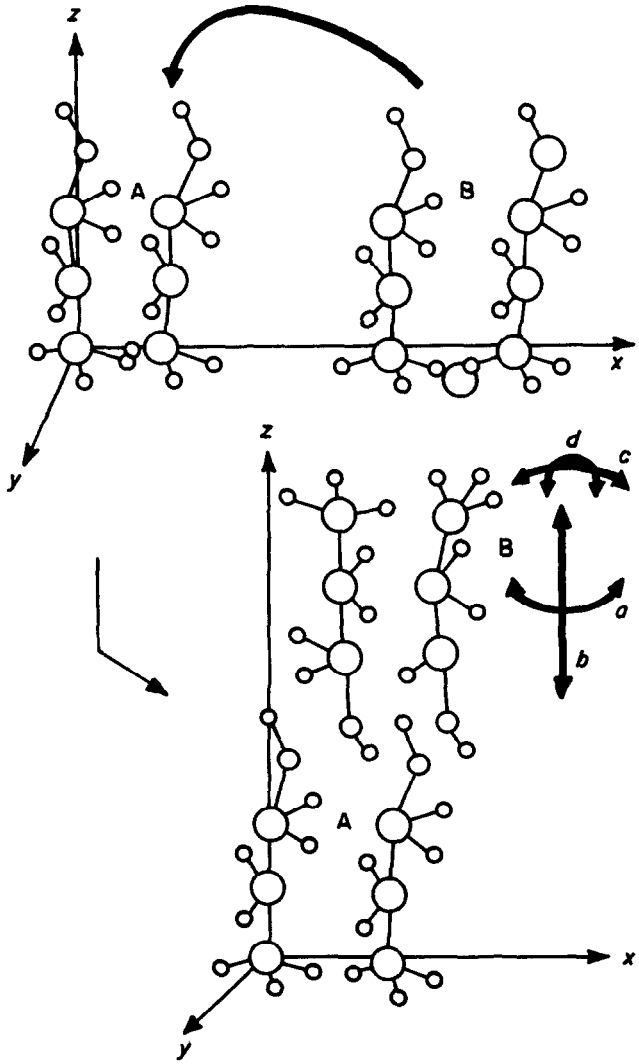

Figure 3. Schematic representation of the packing procedure of lipid molecules assembled in bilayers

ordinates, type of atom, . . .). The PLUTO program[6] was eventually employed for drawing the most probable structure with the Calcomp 1051 drawing table. Figure 5 shows the structure of a lipid, dipalmitoylphosphatidylcholine, drawn by the Olivetti M40. The image was displayed using a colour monitor with a resolution of 640 × 400 addressable points. Two representations were developed:

- first, a skeleton representation (see Figure 5);
- second, a real volume representation (see Colour Plate 1)

The two representations can be drawn very quickly using a microcomputer (1–10 s for display, depending on the number of atoms).

Procedure organization for monolayer of bilayer formation

From the structure obtained for the isolated molecule the molecular structure of the monolayer was calculated (see Figure 6) using the CHAN5 program. The CHANB program was used to calculate the molecular structure of the bilayer. An example of antibiotic–cardiolipin complexes is shown in Colour Plate 2.

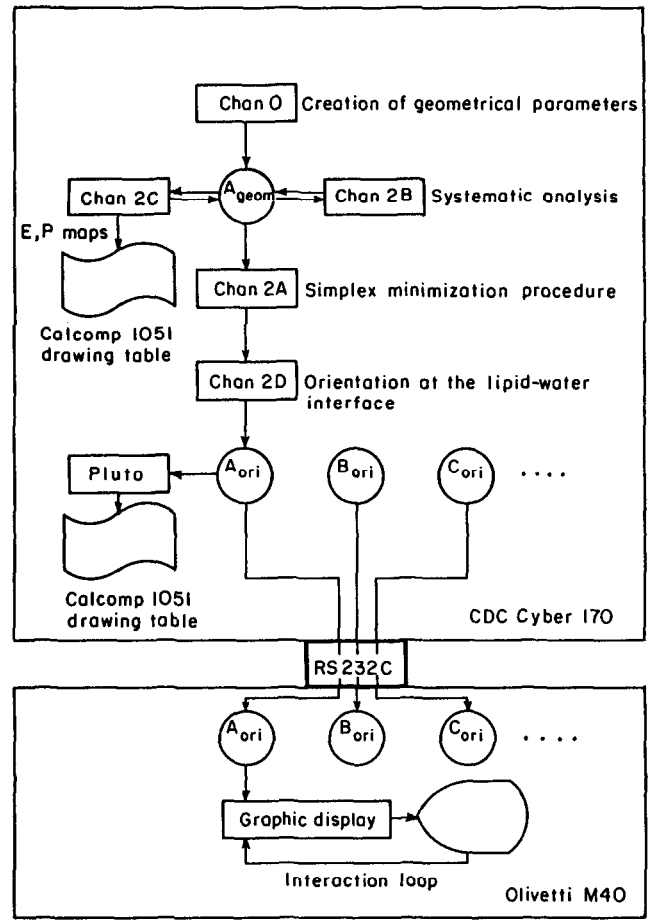

Figure 4. Organization of the procedure for analysis of isolated molecules

CONCLUSION

The date previously reported for many lipids[2,9,10] demonstrates the feasibility of predicting the aggregate structures obtained from molecular conformational analysis, bilayers for dipalmitoylphosphatidylcholine[2,9], micelles for palmitoyllysophosphatidylcholine[9] and inverted micellar structures for dioleoylphosphatidic acid[9] or monogalactosyldiacylglycerol[10]. For dipalmitoylphosphatidylcholine, good agreement has been found between the experimental data and the theoretical

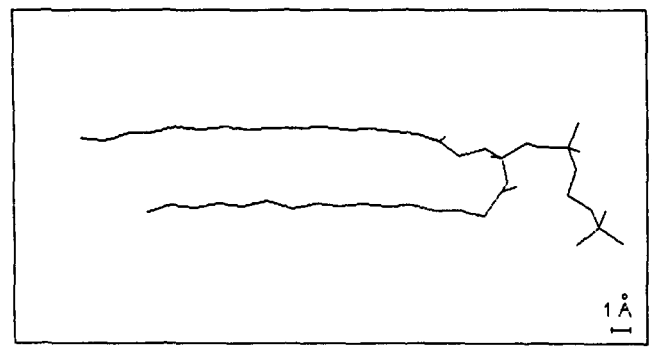

Figure 5. Picture display for dipalmitoylphosphatidylcholine drawn using an Olivetti M40. Picture display is performed using a monitor with 640 × 640 addressable points resolution. There are two types of representation: a skeleton representation (hardcopy) shown here and a real volume representation (see Colour Plate 1)

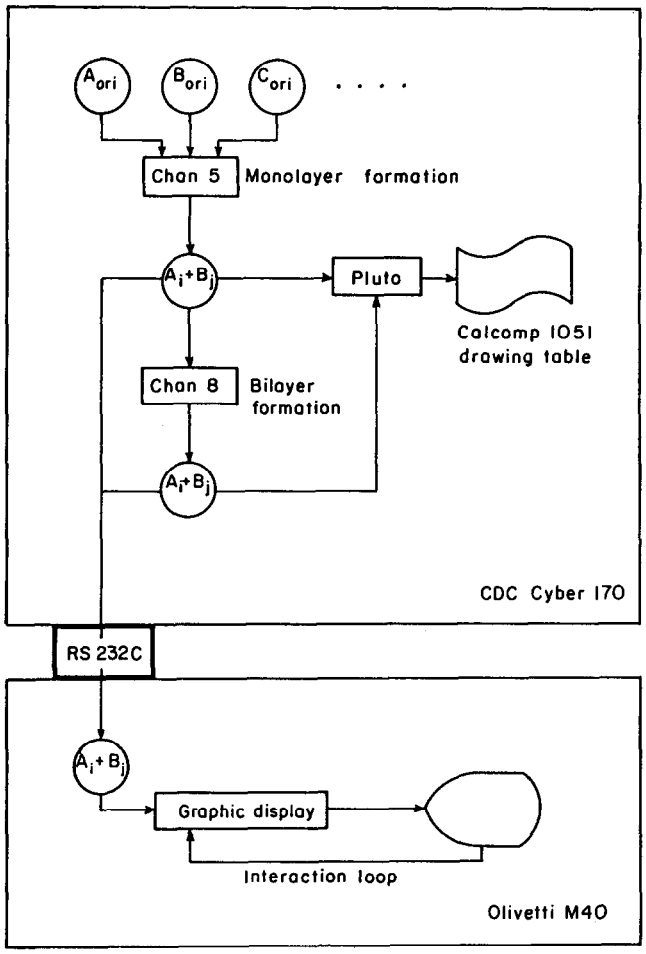

Figure 6. Procedure organization of monolayer and bilayer analysis

predictions. Indeed, neutron diffraction combined with the use of selectively deuterated lipids can provide detailed information about the molecular structure[2]. This approach has recently been applied to bilayer membranes of DPPC deuterated at 12 different positions in the hydrocarbon chain and polar head group. The distances between the centre of the bilayer and each deuterated carbon obtained experimentally and theoretically were in excellent agreement. It can be argued that the conformational analysis interactions between molecules are not allowed to perturb the minimum-energy conformation. However, the interaction energy between molecules can modify each structure's probability as has been demonstrated for dipalmitoylphosphatidylcholine. In this case, the most stable structure was different for the isolated lipid molecule and for the lipid in the monolayer. It was obvious that monolayer packing stabilizes structures characterized by the close proximity of the phosphate residue (associated with the hydrophilic moeity of one lipid) and the choline residue (associated with the adjacent lipid); the electrostatic interaction between the two residues stabilizing the lipid structure. The conformational analysis described here is applicable to the amphiphilic molecules. This procedure has already made it possible to provide a molecular description of the mode of insertion of antibiotics[3,11,12,13], antimycotics[14], tumour promoting agents[15] and chlorophyll *a*[16] into the lipid matrix. It also allows the prediction of the ionophoretic properties of drugs[3,8,11] before their synthesis, and offers a unique way to identify transcient conformations of ionophores crossing lipid membranes. Finally, the TAMMO technique associated with the experimental analysis (neutron diffraction, infrared attenuated total reflection, RX diffraction, . . .) permits the molecular description of the membrane organization.

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