MODE OF ORGANIZATION OF GALACTOLIPIDS : A CONFORMATIONAL ANALYSIS

Robert BRASSEUR, Joëlle DE MEUTTER and Jean-Marie RUYSSCHAERT

1. INTRODUCTION

Galactolipids are major components of thylakoid membranes; monogalactosyldiacylglycerol (MG) and digalactosyldiacylglycerol (DG) comprising about 50% and 25% of the total polar lipids. Native monogalactosyldiacylglycerol forms an hexagonal H_{II} phase under hydratation; a property that has been attributed to its "cone shaped" structure (D.J. MURPHY, 1982; P.J. QUINN, W.P. WILLIAMS, 1983). If several hypothesis have been proposed to explain the abundance of this peculiar organization, its precise role has not yet been elucidated. It is the purpose of this paper to demonstrate that this kind of organization can be predicted from the knowledge of the intra- and intermolecular interactions between lipid molecules. This approach is based on a conformational analysis procedure allowing to calculate the structure of assembled amphiphilic molecules.

2. METHOD

The computational approach supposes a two steps procedure. First, the conformation of the isolated molecule and its orientation at a simulated lipid-water interface is calculated by a method used elsewhere (R. BRASSEUR et al., 1981; R. BRASSEUR et al., 1982_a). Briefly, the total conformational energy was calculated from the Van der Waals, torsional and electrostatic energies. The latter was calculated for a dielectric constant of 16, a value intermediate to those currently used for the aqueous and hydrophobic phases at the simulated interface (R. BRASSEUR et al., 1982_a). Selected conformers were then submitted to a simplex minimization procedure (J.A. NELDER, R. MEAD, 1965) and their orientation at the interface defined from the hydrophobic and hydrophilic gravity centers (R. BRASSEUR et al., 1983; M. DELEERS et al., 1983). In the second step of the procedure, the assemblage of the molecules in the monolayer was computed as described elsewhere (R. BRASSEUR et al., 1981). When the configuration of the cluster has been established, the mean molecular area was evaluated from both the area occupied by each molecule and the intermolecular area which were obtained after projection on the interface plane using a grid of square, each with a 0,1 nm side (R. BRASSEUR et al., 1982_b). Calculations were made on a CDC Cyber Computer coupled to a Calcomp 1051 drawing table.

3. RESULTS AND DISCUSSION

Di- and monogalactosyl di(cis-9,12,15-hexadecatrienoyl) chains present a great number of rotational angles (Fig.1). If all angles are modified by steps of 60°, more than 10^{27} conformers could be obtained. Therefore, the calculation of conformational analysis was performed on 2 different parts of the molecule. In a first systematic study, the torsional angles located in the hydrocarbon chain ($\beta1,\beta2,\beta3,\beta4,\theta3,\gamma1,\gamma2,\gamma3,\gamma4$)were given successive increments of 60°, yielding 6^9 differents conformations (10.077.696) from which 2 structures of maximal probability were selected. In a second systematic study, the angles $\alpha1,\alpha2,\alpha3,\theta1$ for MG and the angles $\alpha1,\alpha2,\alpha3,\alpha4,\alpha5,\alpha6,\theta1$ for DG located in the polar head moeity were given successive increments of 60°, yielding 6^4 different conformations for MG

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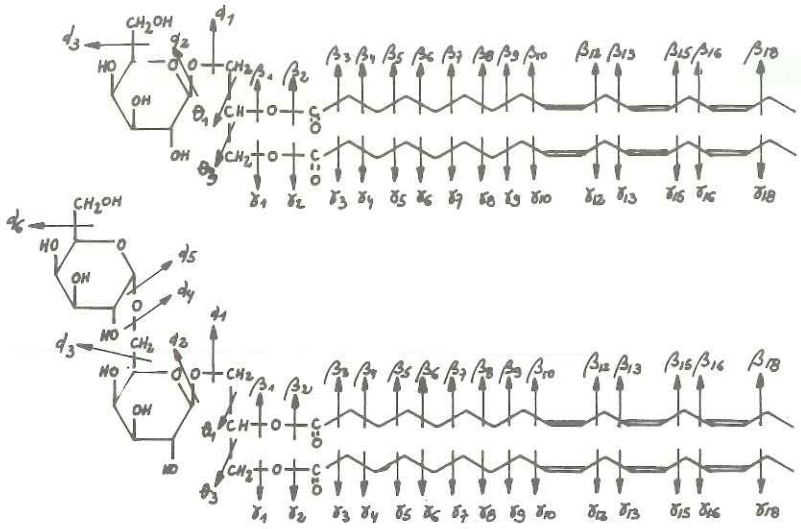


FIGURE 1. Chemical structure and notation of torsional angles in MG and DG.

(1296) and 6^7 different conformations (279 936) for DG. Three for MG and one structure for DG with maximal probability were selected in each case. Combination of the structures obtained for the polar head group and the structures obtained for the hydrocarbon chain gives 6 structures of MG and 2 structures of DG for the entire isolated lipid molecule. The structure of minimum energy was obtained by application of the simplex minimization procedure (J.A. NELDER, R. MEAD, 1965) with a precision of 10° on all rotational angles. Lipid molecules were then assembled in monolayer using a technique described elsewhere (R. BRASSEUR et al., 1981).

As already mentioned, the precise role of the galactolipids has not yet been elucidated. MURPHY (D.J. MURPHY, 1982) proposed recently two possible roles for galactolipids :

a) stabilization of concave curvature regions in thylakoid grana.

b) package of intrinsic membrane proteins inside the bilayer.

Our analysis reveals that formation of inverted micellar structure is possible for MG as a consequence of its calculated "cone shaped" structure. The conformational analysis demonstrated indeed that the area occupied per hydrocarbon chain (96 A^2) is larger than the area occupied per polar head groups (54 A^2). Figure 2 shows that this kind of structure doesn't allow a package in bilayer but that, after assemblage, the structure of the aggregate resembles part of an inverted micellar structure in which the head groups point inward, even if it would be premature to try to define any dimensional parameters associated to these calculated structures in view of the limited number of associated molecules used for the moment in the calculation. The situation is quite different for DG. Indeed, the calculated area for the polar head group (95 A^2) and the hydrocarbon chain (85 A^2) are almost identical and a lipid association in bilayer structure is preferred (Fig.3). This relative position for the double bonds in MG and DG is very surprising and important for the type of organization here described. Indeed, when the same procedure of conformational

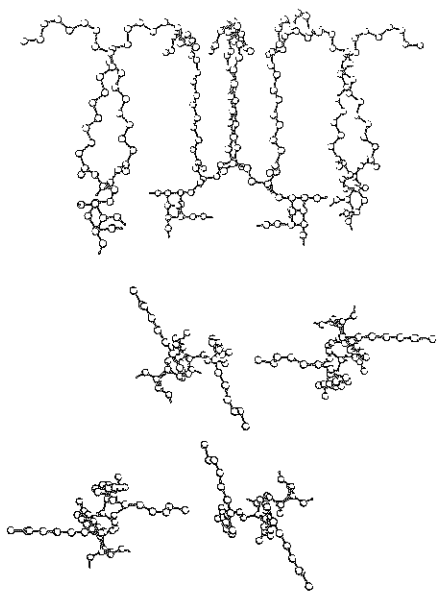


FIGURE 2. Configuration of the monogalactosyldiacylglycerol monolayer. Four molecules of the most probable conformer were assembled and shown in a frontal view (upper) and in an above view (lower).

analysis is applied to a MG molecule in which acyl chains are satured (distearoyl acyl chains), a organization in bilayer structure is obtained (results not shown).

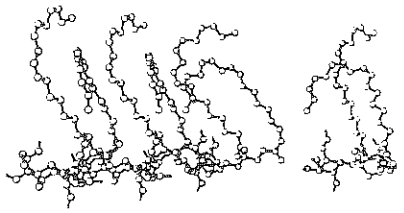
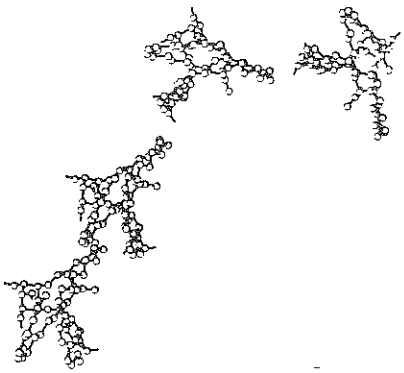


FIGURE 3. Configuration of the digalactosyldiacylglycerol monolayer. Same presentation as in Fig.2.



It is tempting to speculate about the position of 3 double bonds and their orientation rather parallel to the membrane interface. One can imagine that the organization of these bonds can form a conduction layer sandwiched between monolayers of low dielectric constant made of fatty acid acyl chains. The calculated position of chlorophyll a at the interface (to be published) is in favour of this hypothesis. Indeed, the position of the double bond in chlorophyll a would favour an electron flow via this conduction band formed by the association of galactolipid structures. This model based on conformational analysis remain hypothetical even if recent ^{13}C NMR studies reveals a possible association between chlorophyll a and lipids in vivo (K.E. EIGENBERG et al., 1981).

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Authors address : J.M. RUYSSCHAERT, Laboratoire de Chimie Physique des Macromolécules aux Interfaces, Université Libre de Bruxelles, CP 206/2, Boulevard du Triomphe, 1050 Bruxelles, Belgique.