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MODE OF ORGANIZATION OF GALACTOLIPIDS: A CONFORMATIONAL ANALYSIS

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Despite the fact that photosynthetic membranes show the conventional bilayer structure, their major lipid component monogalactosyldiacylglycerol does not form lamellar structure but takes up an hexagonal-II structure when dispersed alone in water and forms inverted lipids micelle structures when dispersed together with other lipid components of the photosynthetic membrane. We present here evidence that the mode of organization of these lipids can be predicted from a conformational approach allowing to describe the configuration of assembled amphiphilic molecules. The minimal conformational energy is calculated as the sum of the contributions resulting from the Van der Waals interactions, the torsional potentials, the electrostatic interaction and the transfer energy. Because of its calculated "cone shaped" structure monogalactosyldiacylglycerol forms inverted lipid structure with the hydrophilic groups pointing inward; for digalactosyldiacylglycerol, an other essential lipid constituent of photosynthetic membrane, its calculated cylindrical shape induces an organization in bilayer structures.

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 (1,2). 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.

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 (3,4). 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 (4). Selected conformers were then submitted to a simplex minimization procedure (5) and their orientation at the interface defined from the hydrophobic and hydrophilic gravity centers (6,7). In the second step of the procedure, the assemblage of the molecules in the monolayer was computed as described elsewhere (3). When the configuration of the cluster has been established, the mean molecular area was evaluated from both the area occupied by

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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 (8). Calculations were made on a CDC Cyber computer coupled to a Calcomp 1051 drawing table.

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 ($\beta_1,\beta_2,\beta_3,\beta_4,\theta_3,\gamma_1,\gamma_2,\gamma_3,\gamma_4$) were given successive increments of 60°, yielding 6^9 different conformations (10.077.696) from which 2 structures of maximal probability (Table I_a) were selected. In a second systematic study, the angles $\alpha_1,\alpha_2,\alpha_3$ θ_1 for MG and the angles $\alpha_1,\alpha_2,\alpha_3,\alpha_4,\alpha_5,\alpha_6,\theta_1$ for DG located in the polar head moeity were given successive increments of 60°, yielding 6^4 different conformations for MG (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 (Table I_b). 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 (Table II). The structures of minimum energy were obtained by application of the simplex minimization procedure (5) with a precision of 10° on all rotational angles. The rotational angles

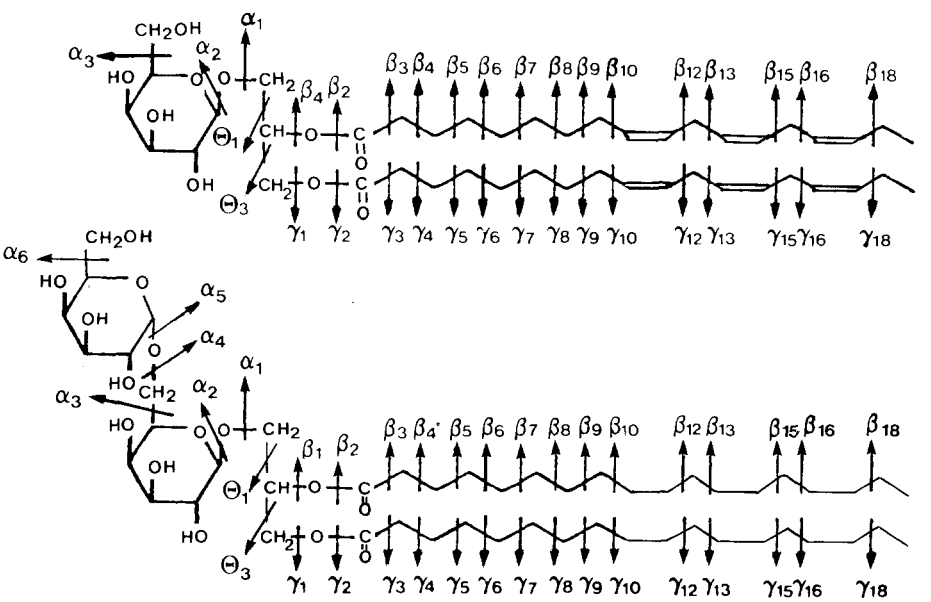

Figure 1. : Chemical structure and notation of torsional angles in MG and DG.

Table Ia : Torsional angles associated to the hydrocarbon chain after systematic analysis

Torsional angles	Conformers	
	e	f
β_1	180	180
β_2	180	300
β_3	300	300
β_4	180	180
θ_3	60	180
γ_1	180	180
γ_2	180	180
γ_3	180	60
γ_4	240	240
Probability %	57	37

of the most probable conformer derived from the latter analysis are given in Table I and lipid molecules were then assembled in monolayer using a technique described elsewhere (3). Our analysis reveals that formation of inverted micellar structure

Table Ib : Torsional angles associated to the MG and DG polar head after systematic analysis

Torsional angles	MG Conformers			DG Conformers
	a	b	c	d
θ_1	120	0	120	240
α_1	180	300	60	180
α_2	120	120	120	120
α_3	180	180	180	180
α_4	-	-	-	300
α_5	-	-	-	180
α_6	-	-	-	180
Probability %	47	33	14	98

Table II: Torsional angles associated to the entire MG and DG molecule after minimization and orientation at the interface. The torsional angles obtained for β and γ with $i \geq 6$ are 180°±5. The precision of the minimization is 10°. The probability was associated to each MG and DG conformer when assembled in monolayer.

	MG Conformers						DG Conformers	
Torsional angles	ae	be	ce	af	bf	cf	de	df
α_1	159	183	42	78	62	71	187	181
α_2	73	93	123	112	121	126	119	116
α_3	178	186	181	184	182	183	172	175
α_4	-	-		-	-	-	290	281
α_5	-	-		-	-	-	182	174
α_6	-	-		-	-	-	180	181
θ_1	77	188	62	207	72	120	240	215
θ_3	65	61	67	60	189	188	184	184
β_1	180	160	189	178	175	176	174	176
β_2	176	178	163	180	294	297	297	301
β_3	300	285	300	293	304	301	297	300
β_4	175	169	177	168	173	169	188	181
β_5	180	170	189	171	181	191	177	181
γ_1	177	181	180	178	175	178	179	166
γ_2	186	180	184	180	175	182	179	191
γ_3	79	63	76	61	63	62	57	299
γ_4	181	235	182	244	264	267	282	243
γ_5	190	189	190	178	174	164	174	173
Probability %	85	4	8	1.5	1.0	0.5	98	2

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 group (54 A^2). Figure 2 shows that this kind of structures doesn't allow a package in bilayer but that, after assemblage, the structure of the aggregates resembles part of an inverted micellar structure in which the head group point inward. 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

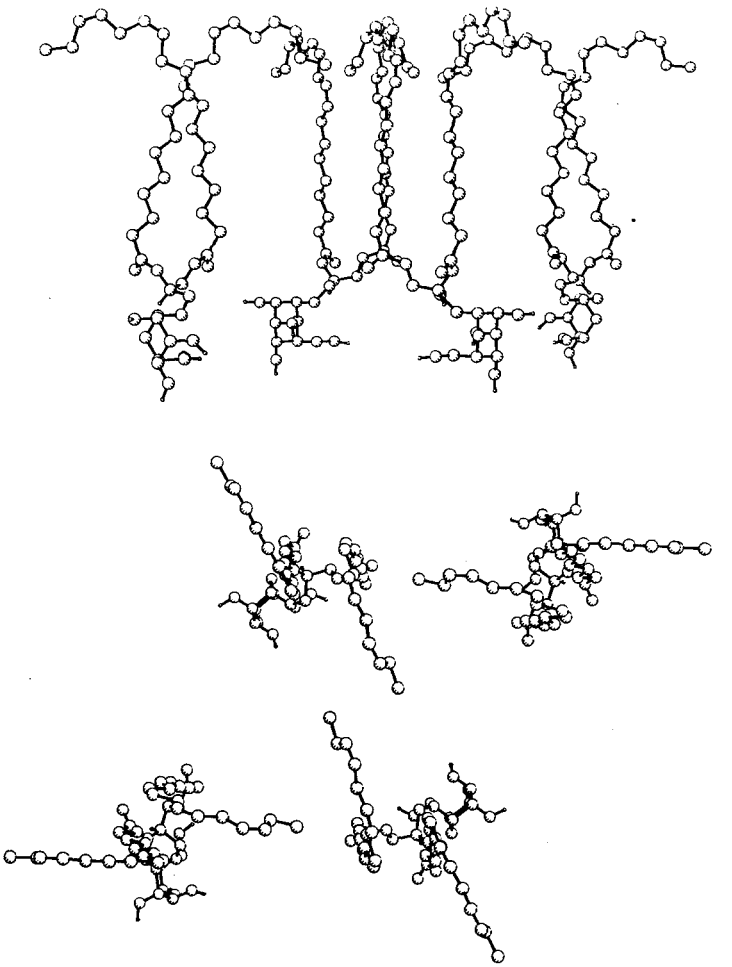

Figure 2. : Configuration of the monogalactosyldiacylglycerol monolayer. Four molecules of the most probable conformer were assembled and shown in a frontal view (upper) and in an above view (lower).

analysis is applied to a MG molecule in which acyl chains are satured (distearoyl acyl chains), a organization in bilayer structure is obtained (Fig.4).

In conclusion, an essential question lies in the role adopted by non-bilayer forming lipids in photosynthetic membranes; their high proportion suggests that they are essential to the maintenance of structure and function of the membranes in some way not yet understood. It would be premature to try to define any dimensional parameters associated to the calculated structure in view of the limited numbers of associated molecules used for the moment in this calculation. It remains however that the procedure allows to predict the phase preference for mono- and digalactosyldiacylglycerol. Murphy (1) proposed recently two possible roles for galactolipids :

1. Stabilization of concave curvature regions in thylakoid grana.
2. Package of membrane proteins inside the bilayer.

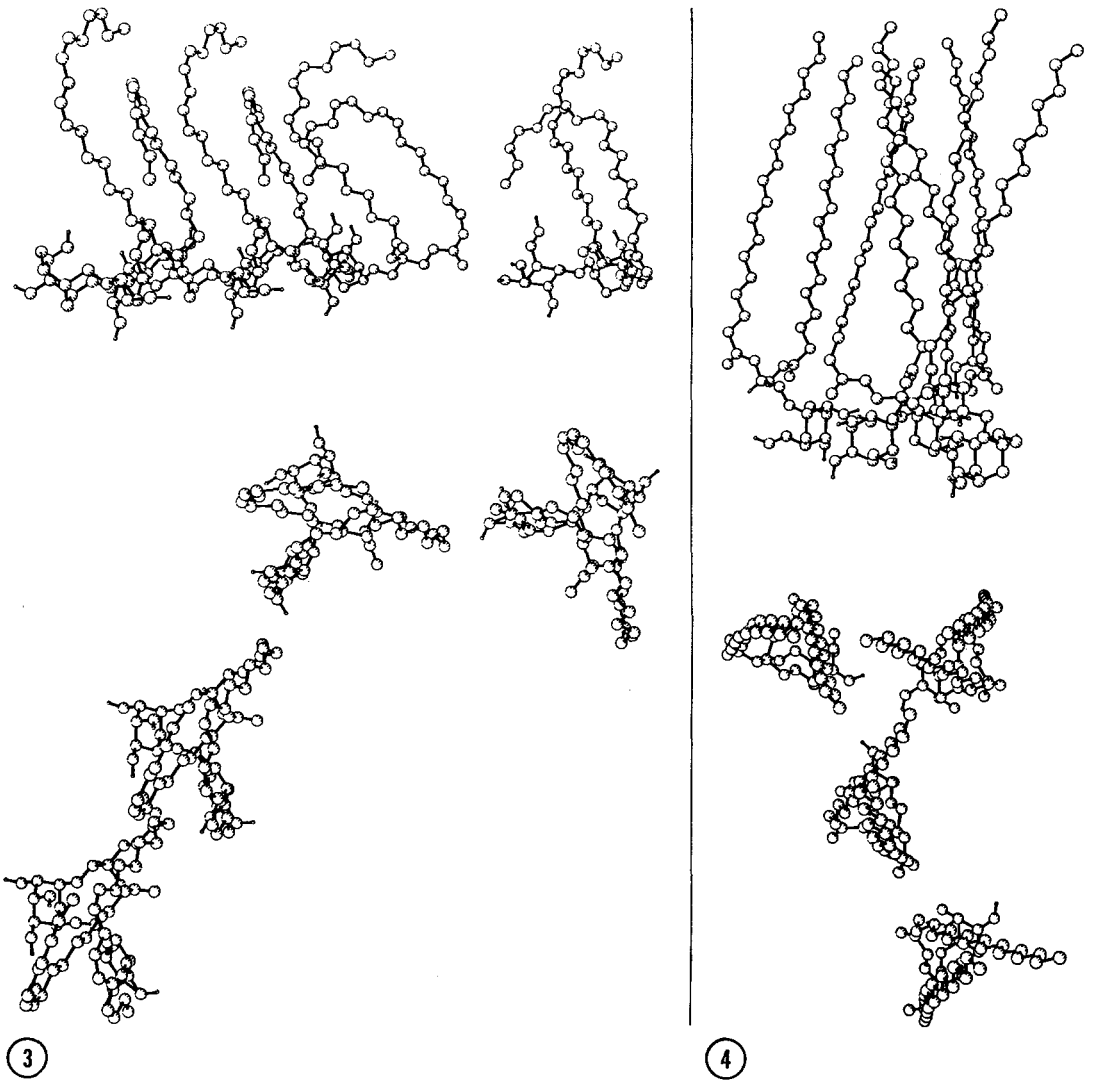

Figure 3. : Configuration of the digalactosyldiacylglycerol monolayer. Same presentation as in Fig.2.

Figure 4. : Configuration of the saturated monogalactosyldiacylglycerol monolayer. Same presentation as in Fig.2.

In the last case, two components of the thylakoid membrane with"inverted cone shaped" structures could, after assemblage permit the formation of a classical bilayer structure. This possibility is presently under investigation in our laboratory for a mixture of mono- and digalactolipid

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