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CONFORMATIONAL ANALYSIS OF MIXED MONOLAYERS OF PHORBOL ESTER AND PHOSPHOLIPID

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SUMMARY : A computational approach was used to study the conformation of mixed monolayers of amphiphilic molecules in order to characterize the assemblage of phospholipids around tumor-promoting and biologically inactive phorbol esters. The theoretical model was in fair agreement with both experimental data obtained in mixed monolayers of phorbol esters and dipalmitoylphosphatidylcholine spread at an air-water interface and the binding of phorbol esters to phospholipid bilayers. Thus, the present method may represent a useful tool to predict the orientation and molecular interaction of amphiphilic drugs at the membrane level.

Several phorbol esters are potent tumor-promoting agents, whilst other phorbol esters are biologically inactive (1-3). The biological response to phorbol esters is thought to involve their binding to membrane receptors (4-7). Several aspects of this specific binding can be mimicked in artificial membranes formed solely of phospholipids (8). Thus, phorbol esters could be first located in the lipid domain of cell membranes and cause a primary disorder in the physico-chemical properties of such a domain (9, 10). Under suitable experimental conditions, there is indeed a close parallelism between the tumor-promoting potency of different phorbol esters and their capacity to either interact with phospholipids (11, 12) or interfere with ionophore-mediated calcium transport in multilamellar lipid vesicles (13, 14). For instance, 4α-phorbol 12,13-didecanoate, which is biologically inactive in terms of either tumor promotion or stimulation of

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insulin release, is much less potent than its biologically active isomer, phorbol 12,13-didecanoate, in displaying repulsive interaction with dipalmitoylphosphatidylcholine (DPPC), in facilitating A23187-mediated calcium exchange-diffusion in multilamellar liposomes formed of DPPC and cholesterol or in inhibiting the binding of [20-^{3}H(N)]-phorbol 12,13-dibutyrate to liposomes formed of egg yolk phosphatidylcholine (12, 14, 8). In order to gain insight into the molecular determinism of such differences, we have previously investigated, by conformational analysis, the configuration and orientation of phorbol esters at a simulated membrane-water interface and their assemblage to form pure monolayers (15, 16). In the present study, we have applied the same procedure to visualize the insertion of distinct phorbol esters within a phospholipid monolayer.

METHODS

A step-wise computation approach was used to predict the configuration of mixed monolayers of DPPC and a phorbol ester. The conformation of isolated molecules and their orientation at a simulated hydrophilic-hydrophobic interface were first established by a method described elsewhere (17, 18). The position of a first molecule of DPPC relative to a molecule of phorbol ester was then assessed by systematic changes of the five following parameters : the distance between hydrophilic centers, the rotation of the lipid molecule around its Z axis, the gravitation of DPPC around the phorbol ester, the up-and-down migration of the lipid molecule and its oscillation around the Z axis. Among the 14,400 possible positions of the DPPC molecule relative to that of the phorbol ester, we selected the position yielding the lowest energy, as judged from both electrostatic and Van der Waals interactions. The position of a second DPPC molecule relative to the selected pair was then calculated in the same manner, and the procedure further repeated until the phorbol ester molecule was completely surrounded by phospholipids. The configuration of the final mixed monolayer was eventually projected either on the interface plane (X,Y) or on a plane perpendicular to the interface. Computations and calculations were made on a CDC-CYBER 170 coupled to a Benson drawing table. The drawing program (PLUTO) was kindly provided by Dr. A. Englert (19).

Four phorbol esters were examined, namely phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-dimyristate (PDM), 4α-phorbol 12,13-dimyristate (4α-PDM) and phorbol 12,13-dibutyrate (PDB). The conformation analysis of the isolated molecule of each phorbol ester was performed as described elsewhere (15). We selec-

ted PDM and 4α-PDM for this study, rather than PDD (phorbol 12, 13-didecanoate) and 4α-PDD, in order to facilitate comparison with PMA, the long acyl chain(s) being identical in PDM, 4α-PDM and PMA, respectively. In pure monolayers, the molecular areas of PDD and PDM were identical, whereas that of 4α-PDM (1.35 nm^2) slightly exceeded that of 4α-PDD (1.15 nm^2).

RESULTS

The assemblage of DPPC molecules around a phorbol ester is illustrated in Figs. 1 (PMA), 2 (PDM) and 3 (4α-PDM), which represent projections on the interface plane. Fig. 4 provides a projection of the 4α-PDM/DPPC mixed monolayer on a plane perpendicular to the interface. In all figures, the carbon atoms in position 15, 16 and 17 were withdrawn from the phorbol backbone in order to increase the legibility of these illustrations.

At each step of the computation procedure, the initial molecule of phorbol ester or the assembly formed by one molecule of phorbol ester and one or more molecule(s) of DPPC was given the

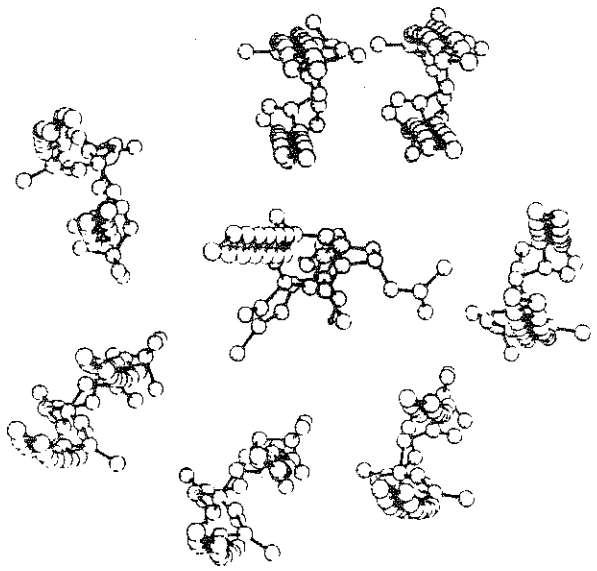

Fig. 1. Projection on the interface plane of a mixed monolayer of PMA and DPPC at a simulated hydrophobic-hydrophilic interface.

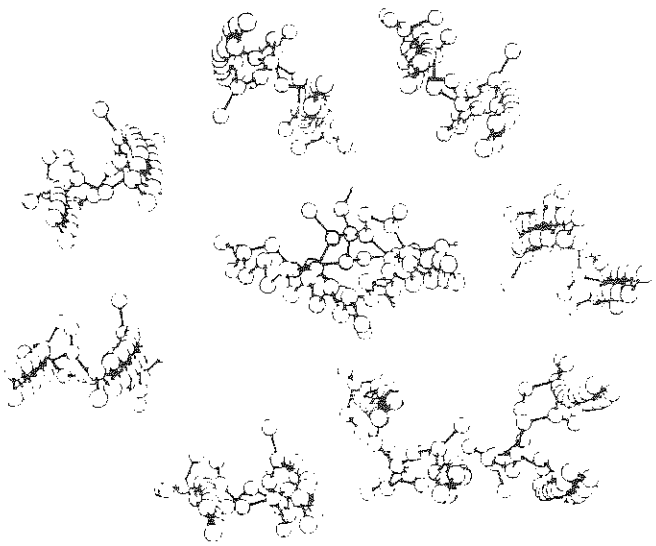

Fig. 2. Projection on the interface plane of a mixed monolayer of PDM and DPPC at a simulated hydrophobic-hydrophilic interface.

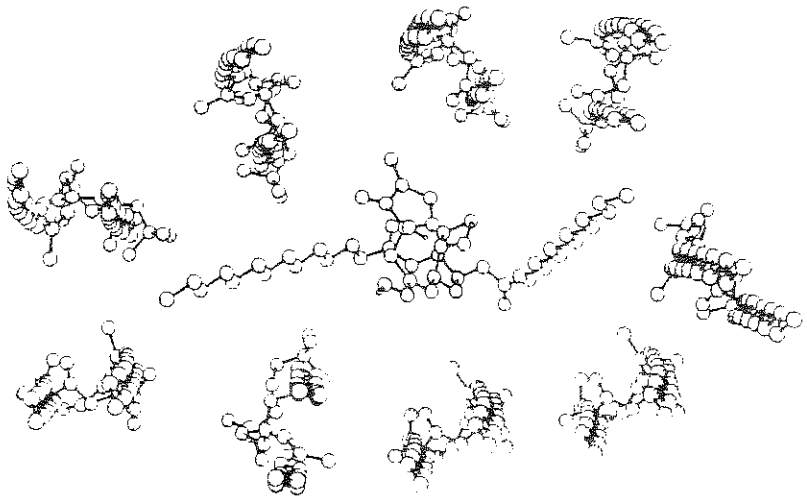

Fig. 3. Projection on the interface plane of a mixed monolayer of 4α-PDM and DPPC at a simulated hydrophobic-hydrophilic interface.

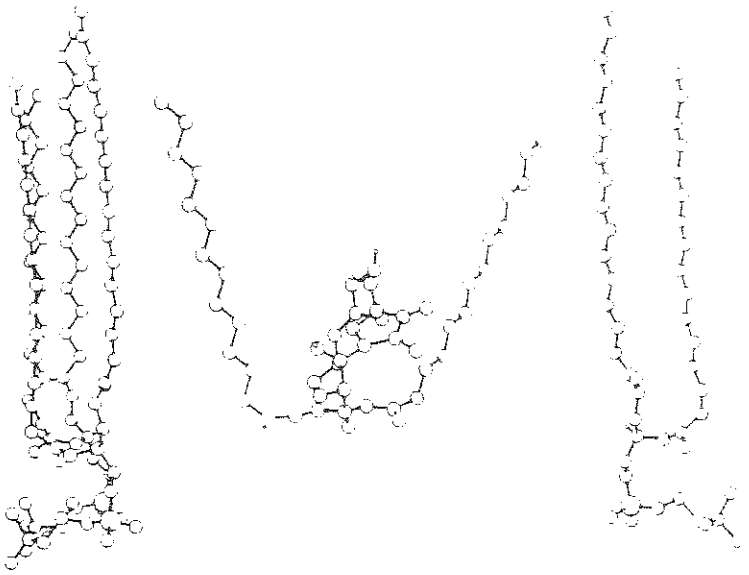

Fig. 4. Projection on a plane perpendicular to the interface of a mixed monolayer of 4α-PDM and DPPC at a simulated hydrophobic-hydrophilic interface. For the sake of clarity, only 3 molecules of DPPC (2 to the left and one to the right) out of the 9 molecules surrounding the 4α-PDM molecule are shown in this projection.

opportunity to interact with either a second molecule of phorbol ester or a further DPPC molecule. In all cases, however, the interaction with DPPC was eventually selected because of a lower energy. This clearly indicates a low probability of existence for monolayers formed of close-to-equal numbers of DPPC and phorbol esters molecules, a situation probably attributable to the repulsive interaction between DPPC and phorbol esters at molar ratio close to unity (12).

The number of DPPC molecules required to completely surround a phorbol ester varied from 6 (PDB) to 9(4α-PDM). The total area of the monolayer assembly, as judged from the area of the polygon joining the hydrophilic gravity center of the DPPC molecules, was not vastly different from that expected from a mere

summation of the individual areas (Table 1), the minor difference suggesting limited attractive interaction. This finding is in good agreement with experimental data collected in mixed monolayers of DPPC and a phorbol ester spread at an air-water interface (12). In such monolayers, a modest attractive interaction between the two molecular species was indeed observed at close-to-physiological surface pressure and at a low phorbol ester/DPPC molar ratio. Incidentally, the phorbol ester/DPPC molar ratio in the mixed monolayers (Figs. 1 to 3) remained well above the experimental value for the binding of phorbol esters to living or artificial membranes. Our model may thus provide a fair view of the insertion of phorbol ester in the phospholipid domain of membranes.

TABLE 1. Mixed monolayers of DPPC and phorbol esters

Molecule	Area in pure monolayers[a]	Number of surrounding DPPC	Theoretical area in mixed monolayers[a,b]	Computed area in mixed monolayers[a]	Cohesion energy[a]
DPPC	0.60	-	-	-	16.0
PMA	0.64	7	2.14	2.11	8.5
PDM	0.69	8	2.49	2.32	15.0
4α-PDM	1.35	9	3.45	3.41	8.5
PDB	0.72	6	1.92	1.83	6.9

[a]Molecular areas are expressed in nm^2 and the mean cohesion energy between adjacent molecules in kcal/mole.

[b]The theoretical area is taken as the sum of the individual areas in pure monolayers corrected for the number of DPPC and phorbol ester molecules in each assembly. In this calculation, the number of DPPC molecules in taken as $(n - 2)/2$, with n representing the number of DPPC molecules completely surrounding each molecule of phorbol ester. This takes into account the facts that (i) the sum of angles in a polygon with n angles equals $(n-2).180°$, and (ii) in imbricated polygones, the sum of angles around each cross point equals 360°.

The cohesion energy in each type of monolayer was due mostly to Van der Waals interaction between adjacent acyl chains rather than to electrostatic interaction between polar heads. From the values for such a cohesion energy, as listed in Table 1, it can be surmised that the insertion of PDM, as distinct from 4α-PDM or PDB, into a monolayer of DPPC is favoured by the attractive interaction between acyl chains. This is in good agreement with experimental data indicating that PDD is about 8 times more efficient than 4α-PDD in inhibiting the binding of tritiated PDB to multilamellar liposomes (8). The cohesion energy in the mixed monolayer of PMA and DPPC was also lower than that found with PDM and DPPC, a situation attributable to the presence of only a single long acyl chain in the PMA molecule.

In conclusion, the present data reinforce the view that the interaction of phorbol esters with phospholipids may play an important role in the expression of their biological potency. Moreover, the present work indicates that the conformational analysis of mixed monolayers formed of distinct amphiphilic molecules may provide useful information to predict the insertion of drugs in the phospholipid domain of biological membranes.

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