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STRUCTURAL ANALOGIES BETWEEN PROTEIN KINASE C ACTIVATORS

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Phorbol esters and diacylglycerols activate protein kinase C but specific structural parameters appear to be required for the enzyme activation. We have analyzed the conformation of potent and not potent diacylglycerols and phorbol esters. The orientation of the CH2OH group at C3 of 1,2 diolein is remarkably similar to that of the same group at C-20 of 4 βphorbol didecanoate and crucial for potency in activating the enzyme. Our data suggest that the new conformational approach here described could be used to rationally design specific inhibitors preventing the effects of tumor promoters and to predict the structure of potential tumor promoters.

Signalling of a large class of extracellular ligands including several hormones, neurotransmitters and mitogens appears to be mediated through Ca^{2+} mobilization and protein kinase C activation (1,2). Upon interaction with specific receptors, the ligand triggers the enhanced breakdown and resynthesis of phosphatidylinositol 4,5 biphosphate resulting in the transient accumulation of diacylglycerol and inositol 1,4,5, triphosphate. The neutral lipid acts as the physiological activator of the protein kinase C, while inositol triphosphate mobilizes Ca^{2+} from its internal store. On the other hand, tumor promoters such as those of the series of phorbol esters, strongly bind to protein kinase C / phospholipid complex and also activate the enzyme (3-5). Both structurally unrelated classes of activators, phorbol esters and diacylglycerols, were shown to compete for the same binding site

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on the kinase (6). Specific structural features, however, appear to be required for the enzyme activation. Indeed 1-2 diolein, the unsaturated diacylglycerol, is by far the best activator when compared with its isomer 1-3 diolein as well as 1-2 and 1-3 distearin, the saturated counterpart (7). Also 4 β phorbol didecanoate is a potent effector while its 4α isomer is devoid of activity (8). A better knowledge of the precise chemical bonding and/or steric interactions involved in activation of protein kinase C is required to rationally design specific inhibitors and predict the structure of potential tumor promoters. We therefore analyzed the conformation of potent diacylglycerols and phorbol esters when inserted into phospholipids and looked for structural analogies. We show that the orientation of the CH_2-OH group at C3 of 1-2 diolein is remarkably similar to that of the same group at C-20 of 4β phorbol didecanoate and crucial for potency in activating the enzyme.

METHODS AND RESULTS

The applied procedure of conformational analysis was based on a strategy currently used for studying polypeptide conformation (9) and modified to describe the mode of assemblage of amphiphilic molecules (10-12). The total conformational energy was calculated as the sum of contributions resulting from the Van der Waals interactions, the torsional potentials, the electrostatic interactions and the energy transfer. The method has been modified in order to take into account the constant dielectric variation at the lipid-water interface. We have assumed a dielectric constant value of 3 at the interface while that of the most deeply water immersed atom was given 30. Between these two values the dielectric constant was assumed to linearly increase along the Z-axis perpendicular to the interface. The most probable ($> 90\%$) structures obtained for each diacylglycerol molecule were minimized according to the

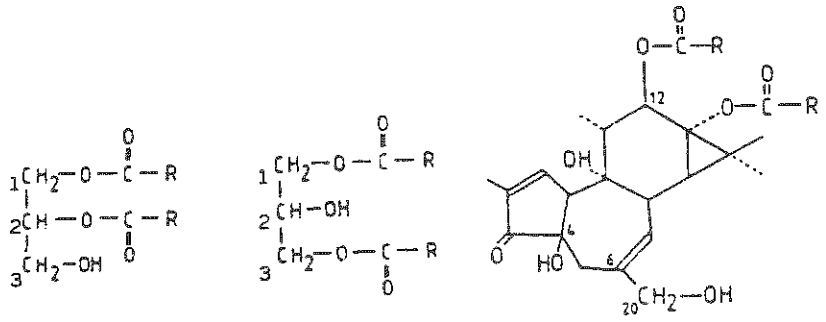
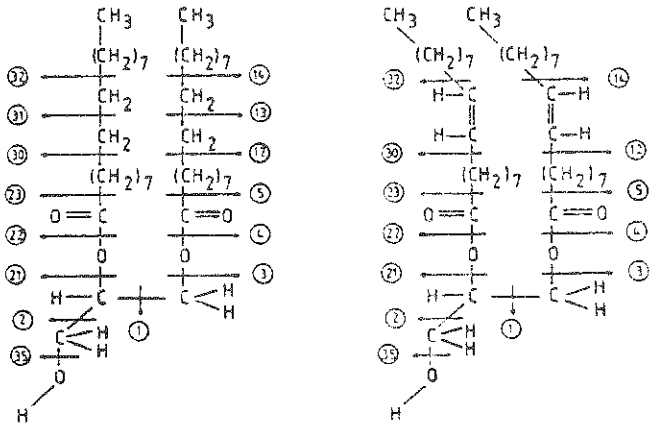


Figure 1. : Chemical structure of diacyl 1,2 and 1,3 glycerols (1,2 DG and 1,3 DG) and phorbol ester.

simplex minimization procedure (13) and then oriented at the interface. The values used for valence angles, bond lengths and atomic charges were those currently used in conformational analysis. Diacylglycerols and phorbol esters (Fig. 1) were inserted into a DL-α-dipalmitoyl phosphatidylcholine (DPPC) monolayer using a procedure described elsewhere (10).

In a preliminary step, the minimum-energy configuration of diacyl 1,2 and 1,3 glycerols, distearin and diolein, was first examined as isolated molecules. Diacylglycerol molecules have 35 rotational angles which give rise to 10^{27} conformers when submitted to systematic 60° changes. The analysis was actually performed on the angles 1, 2, 3, 4, 5, 21, 22, 23 as numbered in Fig. 2, and yields 1,679,616 conformers per molecule. An end view of the most probable conformer of 1,2 distearin, 1,3 distearin, 1,2 diolein and 1,3 diolein is shown in Fig. 3. Then we have determined the most probable association between the diacylglycerols and the DPPC molecules, the conformation of which was previously predicted and experimentally confirmed by neutron diffraction analysis (10). Two parameters describing this association,namely molecular area and interaction energy were calculated. The results given in Table 1 show that the active diacylglycerol form occupies the largest



1,2-DG(18:0) 1,2-DG(18:1)

Figure 2. : Structural formula of 1,2 distearin (1,2 DG 18:0) and 1,2 diolein (1,2 DG 18:1) with numbering of torsional angles.

molecular area and interacts less tightly with the phospholipid neighbourhood. The side views of the series of diacylglycerols esterified with 18-carbon fatty acids as inserted into DPPC monolayer are presented in Fig. 4. For the sake of clarity, phospholipid molecules are not drawn. The values of the torsional angles of the predicted configurations have been calculated and will be exhaustively listed elsewhere. Here we focus on the orientation of the

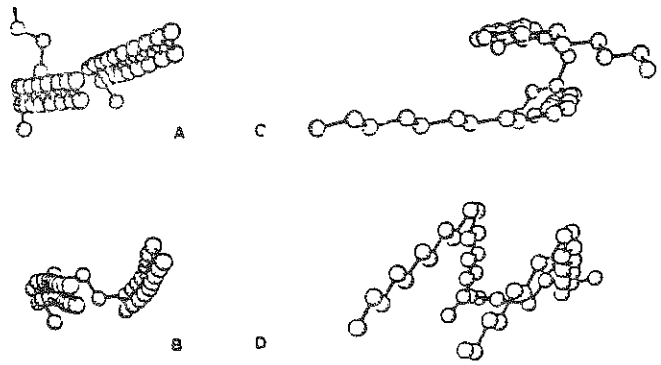


Figure 3. : End views of the most probable conformer of diacylglycerols after minimization procedure and orientation at the lipid/water interface. 1,2 distearin (A) ; 1,3 distearin (B) ; 1,2 diolein (C) ; 1,3 diolein (D). Calculations were performed at 25°C on a CDC-Cyber Computer coupled to a Calcomp 1051 drawing table.

Table 1

Incorporation of diacyl 1,2 and 1,3 glycerols esterified with 18-carbon fatty acid into DL-α-dipalmitoyl phosphatidylcholine (DPPC) : molecular area (A) of diacylglycerol molecule into the lipid monolayer and diacylglycerol/phospholipid interaction energy (E).

Diacylglycerols	A(A^2/mol)	E(Kcal/mol)
1,2 unsaturated	62	-9.8
1,2 saturated	42	-15.3
1,3 insaturated	52	-13.6
1,3 saturated	49	-15.8

Calculations were made as described in the text and the legend to Fig. 3. Mean molecular area and interaction energy of most probable conformations of 1,2 and 1,3 diacylglycerols esterified with saturated 18-carbon fatty acids (distearin) were compared with those of unsaturated 18-carbon fatty acids (diolein) when inserted into DPPC molecule. Molecular area was calculated from the projection on the membrane plane using a grid of 0.1 nm-side squares.

CH_2-OH group at C3 of 1,2 diacylglycerol. The angles formed by C2-C3 (a) and C3-OH (b) bonds with the plane of the lipid/water interface are shown in Fig. 4. Obviously the orientation of CH_2-OH group strongly depends on the nature of the acyl chain since the saturation of the cis-double bond markedly changes the orientation of C2-C3 and C3-OH bonds and unfavours the potency. The CHOH group at C2 yields inactive compounds irrespective of the nature of the chain. To assess the importance of the primary alcohol function, we acetylated the hydroxyl group at C3 of 1,2 diolein. 1,2 dioleyl-3 acetylglycerol was totally unable to activate protein kinase C (data not shown). Similar conformational analysis on phorbol esters has already been reported (14). The molecular structure of 4β phorbol didecanoate (4βPDD) and 4α phorbol didecanoate (4αPDD) as well as the angles formed by the C6-C20 (a) and C20-OH (b) bonds with the lipid/water interface are shown in Fig. 4. The orientation of CH_2-OH group at C20 of phorbol esters

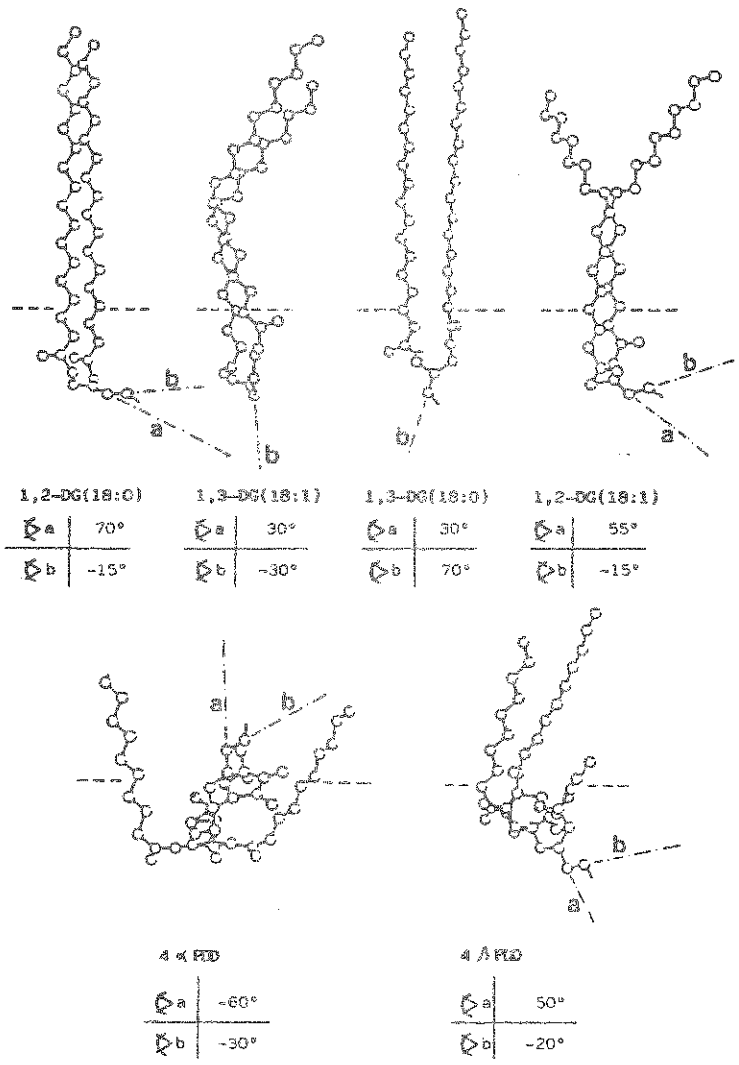


Figure 4. : Molecular arrangement of phorbol ester and diacylglycerols inserted into a DL-α-dipalmitoyl phosphatidylcholine (DPPC) monolayer. The molecules are shown on a side view with the Z axis pointing towards the hydrophobic domain. a and b indicate the orientation of the C2-C3 and C3-OH bonds, respectively, in 1,2 diacylglycerol as well as that of the C6-C20 and C20-OH bonds, respectively, in phorbol esters. The angles formed with the water/lipid interface are displayed for each molecular structure. In the case of diacyl 1,3 glycerol, the mean orientation of C1-C2 and C2-C3 bonds has been taken since both chains in C1 and C3 are not structurally similar. For the sake of clarity the orientation of these bonds has not been represented. The dotted line delineates the lipid-water interface. Calculations for 1,2 distearin (1,2 DG 18:0), 1,3 distearin (1,3 DG 18:0), 1,2 diolein (1,2 DG 18:1) and 1,3 diolein (1,3 DG 18:1) conformations were performed as described in the text and the legend of Fig. 3. Those for 4β phorbol didecanoate (4βPDD) and 4α phorbol didecanoate (4αPDD) conformations were taken from ref. 14.

compared with the interface plane varied greatly like that of the CH_2-OH group at C3 of diacylglycerols. However, the predicted conformations of both classes of compounds reveal a striking similarity between the orientation of the CH_2-OH group at C20 of potent phorbol didecanoate (a = 55° , b = -15°) and that of the same group at C3 of potent diolein (a = 50° , b = -20°). The importance of the primary alcohol group at C20 for biological activity of phorbol esters has already been emphasized since esterification or etherification of this position gives rise to to inactive compounds (15,16).

The present results support the possibility that the hydrophobic domain of diacylglycerols (acyl chains at C1 and C2) and phorbol esters (acyl chain at C12) is required for relatively unspecific interactions with adjacent lipid microenvironment and that, in addition, highly specific electrophilic interactions in volving the CH_2-OH group (at C3 of diacylglycerols and C20 of phorbol esters) are essential for binding to the protein moiety of the protein kinase C/phospholipid complex and subsequent enzyme activation. However the apparent affinity of 1,2 diolein for activating the kinase is two to three orders of magnitude less than that of potent phorbol esters (3) suggesting that additional interactions occurred in the vicinity of the CH_2-OH group in C20 which may confer to the molecule a higher stereospecificity. This is consistent with a recently reported stereochemical model which indicates that the region of the phorbol molecule containing the 3-keto, 4-OH and 20-CH_2OH residues is critical for biological activity of phorbol esters (17). Finally,it is of interest to note that the elucidation of the precise conformation of potent phorbol esters and diacylglycerols when inserted into phospholipids should contribute to design drugs which may be used in preventing the effects of tumor promoters as well as in controlling protein

kinase C-mediated processes such as platelet aggregation, insulin, amylase or histamine release.

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