ADVANCED COMPUTATIONAL DOCKING OF TWO TELEOCIDIN CONGENERS TO CYS2 DOMAIN OF PROTEIN KINASE Cδ

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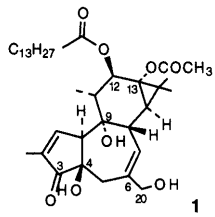
We simulated the docking of two teleocidin congeners to the cys2 domain structure observed in the crystalline complex of protein kinase Cδ with phorbol-13-acetate. The most stable docking models were searched for two conformers of (-)-indolactam-V ((-)-IL-V), twist and sofa form, and for (-)-benzolactam-V ((-)-BL-V8) by using an automatic docking method, ADAM, which can cover all possible binding modes and conformations. The twist form of (-)-IL-V and (-)-BL-V8 molecules fitted well into the same cavity as phorbol-13-acetate. Of the three functional groups hydrogen-bonding to the protein, two hydrogen-bonded with protein atoms in common with phorbol-13-acetate, but the third one hydrogen-bonded with a different protein atom from that in the case of phorbol-13-acetate.

KEY WORDS teleocidin; indolactam; benzolactam; protein kinase C; docking study

Phorbol esters (12-*O*-tetradecanoylphorbol-13-acetate: **1**), teleocidins (*e.g.*, teleocidin B-4: **2**)[1] and (-)-indolactam-V ((-)-IL-V: **3**)[2] are well known to activate protein kinase C (PKC),[3] by binding competitively to the enzyme, although it remains uncertain whether or not this binding is a true trigger of tumor promotion. The relationship between the chemical structures and the activities of these compounds has attracted much attention because of the marked structural dissimilarities. Several research groups have attempted to clarify the structural features essential for the activity by means of molecular superposition of **1** and **2**.[4,5,6] Different superposition models have been proposed, presumably because of the use of different methods, in addition to different molecular configurations and conformations for superposition. NMR study has revealed that both teleocidins and indolactams exist in two stable conformational states with *cis* and *trans* amide conformations. These twist and sofa forms exist in an equilibrium in solution,[7] although a single conformation of each of them was observed in X-ray crystal analyses of teleocidins.[8] The low energy barrier between the two conformers (observed free energy of activation, $\Delta G^{\ddagger}$=18 kcal/mol) left open the question of which is the important conformer for the activity.



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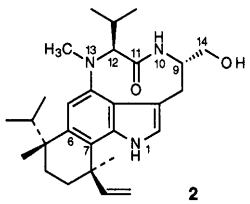

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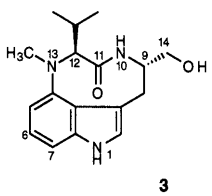

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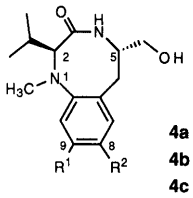

4a R^1 = H, R^2 = H
4b R^1 = *n*-$C_{10}H_{21}$, R^2 = H
4c R^1 = H, R^2 = *n*-$C_{10}H_{21}$

Recently, we have synthesized (-)-benzolactams (*e.g.*, (-)-BL-V8-310: **4b**) as new active congeners having an eight-membered lactam ring instead of the nine-membered lactam ring and a benzene ring instead of the indole ring in indolactams, with hydrophobic substituents. The biological activity of **4b**, in which the *trans*-amide (sofa-like) form is relatively too unstable to be the active conformation, is stronger than that of **3**, clearly indicating that the twist form is the active conformation of teleocidins.[9] In 1995, the crystal structure of PKC δ cys2 domain was elucidated in the complex with phorbol-13-acetate.[10] Docking simulation to this structure would be useful to clarify the relationship between the three-dimensional structures and activities of other PKC activators. Here, we report stable docking models for **3** and (-)-BL-V8 (**4a**: the key structure of **4b** without the *n*-decyl group) to PKC δ cys2 domain, obtained by using an automatic docking program, ADAM, which we have previously developed.[11]

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Method For **3**, dockings of the twist form and sofa form were performed independently. The structures were taken from the crystal structures of teleocidin B-4 and olivoretin A,[8] respectively, by removing the alkyl moieties. All hydrogen atoms were relocated at appropriate positions. For **4a**, only a *cis* amide structure, which was very similar to the twist form of **3**, was modeled on the basis of the NMR spectra and was used for docking. All the flexibilities in rotatable bonds are automatically considered in the docking procedures whereas the protein structure is treated as fixed in the initial docking process. The allowed binding region for compounds to be docked was prepared by removing the phorbol-13-acetate from the crystal structure of PKC δ cys2 domain-phorbol-13-acetate complex taken from the Protein Data Bank (1PTR).

The program ADAM was developed for automatically searching for stable docking models, including the most stable one for a given pair of ligand and protein molecules, covering all possible binding modes and ligand conformations. It can reliably obtain the most stable docking model without any preconception. Promising docking models are selected based on force field energy for van der Waals interaction and electrostatic interaction in the docking process. The characteristic of the method is that ligand torsion angles in the output models are given as continuous values, being repeatedly optimized together with the relative position and orientation of the molecule. The several initial docking models are reranked after energy minimization using the AMBER program,[12] taking into account conformational flexibilities in side chain groups and the presence of water molecules in the active site. In the calculation of electrostatic energy, the dielectric constant ε was assumed to be 4 times the interatomic distance.

Results and Discussion First, the reliability of the ADAM program was tested by redocking phorbol-13-acetate to the PKC δ cys2 domain without any presumption. The most stable docking model output from the program closely resembled the structure of the complex in the crystal, fully reproducing the binding mode and conformation, as shown in Figure 1(a). Three functional groups hydrogen-bond to main chain amide groups in the cavity: C3-carbonyl to the NH of Gly253, C4-OH to carbonyl of Gly253 and C20-OH to both the carbonyl of Leu251 and the NH of Thr242.

Then, the twist form and sofa form of **3** and **4a** were docked to the protein. Features of the most stable docking model for each conformer (or molecule) are as follows. The twist form of **3** fitted well to the cavity occupied by phorbol ester, as shown in Figure 1(b). Three functional groups participated in hydrogen-bonding to the protein as with phorbol ester. The C11-carbonyl hydrogen bonded to Gly253, and C14-OH to the carbonyl of Leu251 and the NH of Thr242; they correspond to the C3-carbonyl and C20-OH groups in phorbol-13-acetate, respectively. However, the

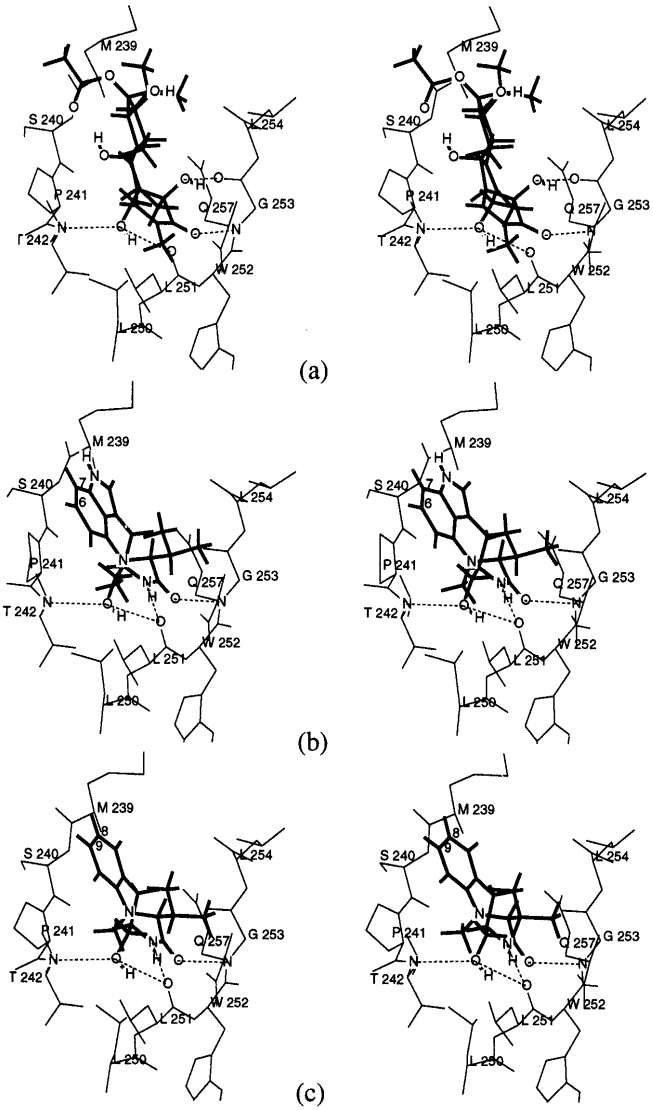

Figure 1 Stereo drawings of the most stable docking models of (a) phorbol-13-acetate (**1**), (b) (-)-IL-V (**3**) twist conformer, and (c) (-)-BL-V8 (**4a**). Bound ligands are shown with bold lines and intermolecular hydrogen bonds (distances less than 3.2 Å) are shown with dotted lines.

partner of amide NH was the carbonyl of Leu251, different from the partner of the third hydrogen-bonding group in phorbol-13-acetate. The large hydrophobic moieties in teleocidins can exist outside the cavity without any steric hindrance in this model. On the other hand, the sofa form molecule could not fit into the cavity, unless large changes occur in the size and shape of the cavity as a result of induced fit. Even in the most stable model, the molecule does not reach the bottom of the cavity due to the close van der Waals contacts. The **4a** molecule also formed a stable complex with the same hydrogen-bonding pattern as the twist form of **3**, as shown in Figure 1(c). The model explains well the facts that the activity of (-)-BL-V8-210 (**4c**: 8-*n*-decyl (-)-BL-V8) is 30 times weaker than that of **4b** and that introduction of a bulkier substituent at C-8, such as a tertiary alkyl group, causes a large decrease of the activity, of the order of 10^3 in the binding assay to PKC δ.[13] Substituent groups at C8 would hinder the fitting of the molecule into the cavity through van der Waals contacts with Ser240 and Met239.

The relationship between the result of molecular superposition study and that of docking study is essentially consistent. By superposing molecules so the hydrogen-bond partner sites expected in the protein coincide between the molecules, we could correctly predict three important functional groups in both phorbol ester and teleocidin. Regarding the active conformation of teleocidin, however, the sofa form was chosen as the more favorable structure. This result provides a good example to show scope and limitation of molecular superposition studies performed in the case where the structure of the receptor protein is unknown: molecules do not necessarily interact with the same functional groups in a protein, even in the case that they bind competitively to a common receptor. Docking study seems to be more reliable than molecular superposition, because of the use of definite structural information on the receptor protein. However, the result may still not be reliable, unless the most stable docking model is searched by an efficient docking method, which can cover all possible binding modes and ligand conformations automatically. Conventionally, docking studies are performed interactively on computer graphic displays. Such studies are likely to favor models arbitrarily searched or those searched with preconception, and they are not likely to give the most stable docking model reliably and reproducibly among the huge number of possibilities.

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