Molecular Models of N-Benzyladriamycin-14-valerate (AD 198) in Complex with the Phorbol Ester-Binding C1b Domain of Protein Kinase C-δ

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N-Benzyladriamycin-14-valerate (AD 198) is a semisynthetic anthracycline with experimental antitumor activity superior to that of doxorubicin (DOX). AD 198, unlike DOX, only weakly binds DNA, is a poor inhibitor of topoisomerase II, and circumvents anthracycline-resistance mechanisms, suggesting a unique mechanism of action for this novel analogue. The phorbol ester receptors, protein kinase C (PKC) and β2-chimaerin, were recently identified as selective targets for AD 198 in vitro. In vitro, AD 198 competes with [3H]PDBu for binding to a peptide containing the isolated C1b domain of PKC-δ (δC1b domain). In the present study molecular modeling is used to investigate the interaction of AD 198 with the δC1b domain. Three models are identified wherein AD 198 binds into the groove formed between amino acid residues 6-13 and 21-27 of the δC1b domain in a manner similar to that reported for phorbol-13-acetate and other ligands of the C1 domain. Two of the identified models are consistent with previous experimental data demonstrating the importance of the 14-valerate side chain of AD 198 in binding to the C1 domain as well as current data demonstrating that translocation of PKC-R to the membrane requires the 14-valerate substituent. In this regard, the carbonyl of the 14-valerate participates in hydrogen bonding to the δC1b while the acyl chain is positioned for stabilization of the membrane-bound protein-ligand complex in a manner analogous to the acyl chains of the phorbol esters. These studies provide a structural basis for the interaction of AD 198 with the δC1b domain and a starting point for the rational design of potential new drugs targeting PKC and other proteins with C1 domains.

Introduction

The anthracycline antibiotics are among the most effective agents used for the treatment of cancer.1 It is generally accepted that the anthracyclines, exemplified by doxorubicin (DOX; Figure 1), exert their antitumor effects through DNA binding and inhibition of DNA topoisomerase II (topo II).2 N-Benzyladriamycin-14-valerate (AD 198; Figure 1) is a novel semisynthetic analogue produced through efforts to modulate anthracycline-DNA binding properties.3,4 AD 198, unlike DOX, only weakly binds DNA and is virtually devoid of topo II inhibitory activity.5-7 Despite these mechanistic differences, AD 198 retains potent cytotoxic activity and circumvents multiple forms of DOX resistance.8-10 In vivo AD 198 is hydrolyzed to N-benzyladriamycin (AD 288; Figure 1) by cellular esterases.11 However, AD 288 has a pharmacological profile similar to that of DOX and fails to elicit the same biological responses observed with AD 198 exposure.3,5,7 Thus, it is unlikely that AD 198 is a prodrug for AD 288. These differences as well as the finding that AD 198 lacks the dose-limiting cardiac toxicity associated with DOX and other anthracyclines suggest that the synthetic modifications incorporated into the AD 198 structure have conferred an atypical mechanism of action upon this compound.

We recently identified protein kinase C (PKC) and the novel phorbol ester binding protein, β2-chimaerin, as molecular targets selective for AD 198.12 In vitro, AD 198 inhibits the kinase activity of the phorbol ester-responsive classical and novel isoforms of PKC in a
competitive manner with respect to ligands for the phorbol ester binding domain. AD 198 competes with [3H]phorbol-12,13-dibutyrate (PDBu) for binding to PKCs ($K_i = 2.5 \pm 0.2 \mu M$ for PKC-δ and $2.8 \pm 0.3 \mu M$ for rat brain PKC) and β₂-chimaerin ($K_i = 1.5 \pm 0.2 \mu M$), a non-kinase phorbol ester receptor. Furthermore, AD 198 competes with [3H]PDBu for binding to the isolated C1b domain of PKC-δ (δC1b, $K_i = 2.7 \pm 0.2 \mu M$) demonstrating that a single C1 domain is sufficient for an interaction with AD 198. The phorbol-unresponsive PKC-ζ, on the other hand, is not affected by AD 198. These values are consistent with PKC as a likely primary target for the cytotoxicity observed against CEM human lymphoblastic leukemia (IC$_{50} = 8.0 \pm 1.0 \mu M$), although comparison of $K_i$ and IC$_{50}$ concentration is difficult as the drug is highly lipophilic and probably active in the membrane.

The δC1b binds phorbol esters and other ligands within a groove formed between the strands of a “pulled apart” β-sheet motif. The crystal structure of phorbol-13-acetate in complex with the δC1b domain has provided a starting point for extensive molecular modeling studies investigating the interactions of diverse ligands with this domain. The results from these studies have been successfully applied to the design of novel ligands for PKC and other phorbol ester receptors.

Using similar techniques, we have modeled the interactions of AD 198 with the δC1b domain. We report three theoretical models for the interaction of AD 198 with the δC1b domain of which one is most consistent with available experimental data. The implications of these models with respect to the activity of AD 198 and in the design of novel anticancer drugs are discussed.

Materials and Methods

Nonstandard Abbreviations: DOX, doxorubicin, Adriamycin; AD 198, N-benzyladriamycin-14-valerate; AD 32, N-trifluoracetyladriamycin-14-valerate, valrubicin, Valstar; AD 288, N-benzyladriamycin; AD 48, adriamycin-14-valerate; PKC, protein kinase C; PDBu, phorbol-12,13-dibutyrate; PMA, phorbol-12-myristate-13-acetate; DAG, diacylglycerol; DOG, dioctylglycerol; cPKC, classical PKC isoform; nPKC, novel PKC isoform; aPKC, atypical PKC isoform; topo II, DNA topoisomerase II.

Molecular Modeling Studies. The structures for AD 198, AD 288, and DOX were generated using the “Small Molecule Builder” application within the Molecular Operating Environment (MOE)$^{19}$ program and minimized using the AMBER94 force field.$^{20}$ The AMBER94 force field was selected over other force fields implemented in the MOE program for two reasons. First, it was developed for protein simulations. Second, geometry optimization of a model anthracycline ring system resulted in a planar geometry when the AMBER94 force field was used, whereas both the MMFF94 and AMBER99 force fields implemented in MOE version 1998.10 gave a twisted chromophore. All calculations were carried out with a distance-dependent dielectric constant of 1 on structures previously minimized to a 0.001 root-mean-square gradient (RMSG). Conformers of AD 198 were generated using the random incremental pulse search (RIPS) methodology within MOE. RIPS adds random increments to atomic coordinates followed by geometry optimization to generate random conformations. The coordinates for the complex of phorbol-13-acetate and the PKC-δ C1b were downloaded from the Protein Data Bank (PDB), accession code 1ptr.$^{13}$ The PDB entry includes no crystallographic water molecules or unknown atoms. The complex was minimized with the AMBER94 force field to a 0.001 RMSG after the addition of hydrogen atoms. Docking of AD 198 was carried out by hand or using the automated docking module, MOE-DOCK, of MOE. Manual docking involved rigid translation of selected conformations of AD 198 relative to the C1b domain to reach a position that maximized interactions with residues known to influence binding on the basis of mutagenesis studies. The choice of conformations for this procedure is described in the Docking Studies subsection of the Results section. MOE-DOCK was validated by removing phorbol-13-acetate from the crystallographic complex and docking it back in. Seventeen complexes were generated in the validation process from random initial positions and conformations. The complex ranked as most favorable was geometry optimized to a 0.01 RMSGs and compared back to the crystallographic complex. The RMSD over all atoms was less than 1.5 Å. In fact, 3 of the 17 complexes had RMSD values less than 2.0 Å on comparison to the crystallographic position. Complexes of AD 198 and the PKC-δ C1b domain were refined using molecular dynamics simulations carried out as follows: structures were first heated to 300 K for 10 ps and equilibrated for 10 ps at this temperature, and data collection was performed throughout the subsequent 100 ps. The dynamic trajectories from these simulations were analyzed by taking snapshots of the system every picosecond during the final phase of the simulation, yielding 100 snapshots. These snapshots were minimized to a 0.001 RMSG prior to analysis. The orientations of the N-benzyl and 14-valerate moieties were further refined using hybrid Monte Carlo simulations. Polar solvent-accessible surface areas were computed using the access program$^{21}$ and averaged over the snapshots taken during the molecular dynamics simulation. Complexes of AD 288 and DOX were generated by using the lowest-energy-minimized snapshots obtained from the refinement of the AD 198 complexes with the same refinement and analysis procedures. The strength of hydrogen bonds ($E_{HB}$) formed between the protein and ligand were measured using the following equation as described recently by Kozikowski et al.$^{22}$

$$E_{HB} = \left( A - Br^2 - 2Br \cos(\theta) \right) \times \cos(\theta_{AA-A-H}) - \cos(\theta_{AA-A-H+p})$$

This equation provides a quantitative analysis of the strength of hydrogen bonds based upon the following component atoms; AA, A, H, and D (acceptor antecedent, acceptor, hydrogen, and donor heavy atom) and their optimal geometries. $r_{AD}$ is the distance between the acceptor and donor. $\theta_{AA-A}\$ is the angle formed by acceptor, hydrogen, and donor. $\theta_{AA-A-H}$ is the angle formed by acceptor antecedent, acceptor, and hydrogen; its optimal value ($\theta_{AA-A-H+p}$) is 120°. $A$ and $B$ are set to 5832 and 972, respectively, as originally reported.$^{22}$ Figures were generated using the molecular rendering package Midas Plus$^{23}$ or by screen shots from MOE.

Translocation Studies. Hydrochloride salts of the DOX congeners (see Figure 1) were prepared as previously described.$^{2,7}$ Translocation studies were carried out using the method of Szallasi et al.$^{24}$ Briefly, subconfluent NIH3T3 fibroblasts were treated with AD 198, N-benzyladriamycin or DOX as indicated in each figure. Cells were disrupted by sonication and fractionated into soluble and membrane fractions. Equal amounts of protein, 5 µg, from each fraction were separated by SDS-PAGE electrophoresis and transferred to nitrocellulose. Blots were blocked with fat-free milk and probed with primary antibodies for PKC-α [mouse, Upstate Biotechnology, Inc. (UBI), New York], 0.5 µg/mL, or PKC-δ, 1 µg/mL (rabbit; UBI) for 2 h at room temperature. Blots were washed with PBS and incubated with a 1:5000 dilution of goat anti-mouse (UBI) for PKC-α and a 1:4000 dilution of goat anti-rabbit (UBI) for PKC-δ conjugated to horseradish peroxidase. Blots were developed using chemiluminescence as described by the manufacturer’s protocol (Pierce). Results are representative of several independent experiments.

Results

C1 Ligands and Membrane Interactions. C1 ligands are amphipathic molecules that interact with...
their receptors, either PKC or other phorbol binding proteins, at the aqueous–lipid interface of phospholipid membranes. A polar region of the ligand interacts with components of the aqueous interface, i.e., lipid headgroups and water, while also providing hydrogen-bonding partners for amino acid residues within the binding pocket of the C1 domain. Hydrophobic moieties of the ligand serve to position the ligand within the membrane as well as to anchor the ligand–receptor complex to the membrane. These characteristic features are demonstrated for phorbol-12-myristate-13-acetate in Figure 2A.

As a starting point for our modeling studies, the conformational space available to AD 198 was investigated. The RIPS conformational analysis was chosen because of its ability to quickly and efficiently sample the conformational space of small molecules containing a large number of rotatable bonds. As anticipated, the 14-valerate side chain and the N-benzyl function of AD 198 are highly flexible. The low-energy conformers of AD 198 generated from the RIPS analysis were screened for the typical amphipathic characteristics of C1 ligands. A low-energy conformation of AD 198 was identified in which the hydrophobic anthraquinone ring system, N-benzyl group, and 14-valerate side chain are oriented above an arbitrary plane below which polar oxygen atoms are present for interaction with the aqueous phase and for hydrogen bonding to the C1b domain (Figure 2B). Importantly, this theoretical membrane orientation is consistent with previous studies of AD 198 interactions with the phospholipid bilayers.

**Docking Studies.** Previous investigations have identified a triangular arrangement of heteroatoms common to diverse classes of PKC ligands assumed to be critical for receptor binding. In the crystal structure of phorbol-13-acetate, only two of these positions, the heteroatoms at C3 and C20, are directly involved in binding to the δC1b. This discrepancy led to questions regarding the validity of the crystal structure. However, it is now thought that the missing pharmacophore elements reflect interactions of the ligand with lipid headgroups or water molecules at the lipid–aqueous interface which are absent in the crystal complex.

The conserved heteroatom arrangement of C1 ligands led us to use a pharmacophore-guided approach for the initial docking simulations of AD 198 to the δC1b. A putative three-point pharmacophore similar to that of the phorbol esters and DAG was identified for AD 198. As shown in Figure 3, this pharmacophore arrangement gives a good fit for AD 198. However, a direct overlay of AD 198 upon the equivalent positions of phorbol-13-acetate bound to the δC1b domain results in a severe steric clash due to the orientation of the anthraquinone ring system into the interior of the protein (not shown). The steric clash was manually adjusted and the resulting complex designated as model A (Figure 4A). Model A was minimized and refined using molecular dynamics simulations.

In the refined model A, only two of the putative pharmacophore elements are involved in binding throughout the dynamic simulations. The most stable of these interactions is the hydrogen bond formed between the C15 carbonyl of AD 198 and the amide hydrogen of Gly 23. During the 100-ps simulation, hydrogen bonds are formed transiently between the C11 phenolic oxygen and the C12 carbonyl oxygen to the side chain amide protons of Glu 27. Model A is most notable for the hydrophobic interactions between the conjugated anthraquinone ring system and the walls forming the groove of the binding pocket. Interestingly, as shown in Figure 4, the dimensions of the binding pocket correspond well to the three-dimensional shape of the anthraquinone. The marked dynamic instability and paucity of hydrogen bonds are significant weaknesses of this model. The average hydrogen-bonding interactions for model A are summarized in Table 1, and the average hydrophilic solvent-accessible surface area for residues likely to be involved in membrane insertion are shown in Table 2.

Previous modeling studies suggest that ligands for the phorbol binding domain need not interact with the receptor through an identical binding mode. Therefore, a study limited by considerations of only the established pharmacophore may fail to identify the correct binding mode. Manual docking and an automated docking program, MOE-DOCK, were used to avoid this potential limitation. Exhaustive manual docking with the low-energy conformation of AD 198 (Figure 2B) followed by minimization and molecular dynamics simulations led to the identification of model B (Figure 4B).

In model B, the hydrophobic anthraquinone ring system, N-benzyl group, and 14-valerate side chain are oriented into the space above the C1 domain thought...
to be occupied by the membrane in the ternary receptor–ligand–membrane complex. Additionally, the 14-valerate acyl chain is positioned to make hydrophobic contacts with the side chain of Leu 24. Model B, unlike model A, has an extensive hydrogen-bonding network maintained throughout the molecular dynamics simulation (Figure 5). The C15 carbonyl oxygen forms a hydrogen bond to the amide proton of Gly 23, and the C9 hydroxyl hydrogen bonds to the carbonyl of Leu 21. The C11 phenolic proton is a hydrogen bond donor to the carbonyls of both Leu 24 and Tyr 8, while the C11 phenolic oxygen is an acceptor for a side chain amide proton of Gln 27. The importance of the 14-valerate in these interactions may account for the observed selectivity of PKC for AD 198 over AD 288 or DOX in vitro.

The interactions between the δC1b and AD 198 in this model are summarized in Tables 1 and 2.

### Table 1. Averaged Hydrogen-Bonding Interactions between AD 198 and δC1b

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<th>model</th>
<th>hydrogen bond locations</th>
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<tr>
<td>A</td>
<td>C15O HN Gly 23</td>
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</tr>
<tr>
<td></td>
<td>C11O NH Gln 27</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>C12O NH Gln 27</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>sum = 2.8</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>C15O HN Gly 23</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>C9OH CO Leu 21</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>C11OH CO Leu 24 &amp; Tyr 8</td>
<td>0.7 &amp; 0.6</td>
</tr>
<tr>
<td></td>
<td>C11O NH Gln 27</td>
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<tr>
<td></td>
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<tr>
<td>C</td>
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<td>3.2</td>
</tr>
<tr>
<td></td>
<td>C11OH CO Leu 21</td>
<td>1.9</td>
</tr>
<tr>
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<tr>
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### Table 2. Averaged Solvent-Accessible Polar Surface Areas (PSA) and Hydrogen-Bonding Interaction Energies (HB) for AD 198, AD 288, and DOX in Complex with δC1b

<table>
<thead>
<tr>
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<th>DOX</th>
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<tr>
<td></td>
<td>PSA (Å²)</td>
<td>HB (kcal/mol)</td>
<td>PSA (Å²)</td>
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<tr>
<td>A</td>
<td>195</td>
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<tr>
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<td>187</td>
</tr>
<tr>
<td>C</td>
<td>181</td>
<td>7.8</td>
<td>208</td>
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</table>

Figure 4. Models A, B, and C.

Figure 5. Model B: hydrogen bonding.

Automated docking with flexible ligands is a powerful tool for the identification of binding modes in the absence of experimental structural data and has been used successfully with the δC1b domain. In the current study, automated docking was used to supplement the unavoidable limitations of manual docking. Over 100 putative binding modes for AD 198 were identified using the MOE-DOCK module, which we have verified can dock phorbol-13-acetate within a 2.0 Å RMSD of the crystallographic position 3 of 17 times starting from random initial positions and conformations. These AD 198 complexes were manually screened for those meeting the putative ligand–membrane orientation requirements described for model B. Specifically, modes were rejected if the ligand was oriented...
such that hydrophobic interactions with the protein were maximized without hydrogen bonding. A single novel binding mode, model C, was selected and refined using molecular dynamics (Figure 4C). In model C, hydrogen bonds are formed between the amide proton of Gly 23 and the C12 carbonyl oxygen of the C ring as well as between the C11 phenolic hydrogen and the carbonyl of Leu 21. The C9 hydroxyl of AD 198 is hydrogen bonded to the carbonyl oxygen of Ser 10 and transiently to the side chain amide protons of Gln 27 throughout the simulation. As in the other models, the anthraquinone ring makes hydrophobic contacts with Leu 24. The alkyl side chain of Leu 24 fits well within the angle formed between the daunosamine and the B ring of the anthraquinone, while the side chains of Val 25, Met 9, and Gln 27 are in close contact with the anthracycline A ring. In contrast to model B, the 14-valerate does not interact directly with the C1b domain in model C. However, both the N-benzyl group and the 14-valerate are oriented to interact with the putative membrane region above the C1 domain. The specific hydrogen-bonding interactions and solvent-accessible polar surface area for model C are shown in Figure 6 and summarized in Tables 1 and 2.

**Correlation with Biological Data.** We previously demonstrated that the 14-acyl side chain of AD 198 was required for the interaction of anthracyclines with PKC in vitro and that maximum activity is observed with the five-carbon valerate side chain. In intact cells, the interaction of C1 ligands with PKC isoforms results in protein binding to cellular membranes. To further explore the importance of the 14-valerate side chain in the binding of anthracyclines to C1 domains, the ability of AD 198, AD 288, and DOX to promote membrane binding of PKC-α and PKC-δ in NIH/3T3 fibroblasts was investigated. As shown in Figure 7 for PKC-α, AD 198 promotes the membrane binding of both PKC-α and PKC-δ in a dose-dependent manner while neither AD 288 nor DOX elicits this response. These results confirm the importance of the 14-valerate substitution in C1 domain binding also observed in vitro and further corroborate the unique interaction of AD 198 with the C1 domains of PKC isoforms. To quantitatively compare these translocation data with the modeling data, models B and C were used to generate complexes of the ΔC1b with AD 288 and DOX. The complexes with AD 288 showed decreased average hydrogen bond energies in both models as did DOX in model C, Table 2. DOX in model B, however, showed stronger hydrogen bonding than the same model of the AD 198 complex. More relevant for comparison to the translocation data, however, is a measure of the ability of the complexes to interact and insert into the membrane. Palm and Clark have demonstrated that polar surface area is a more important metric than nonpolar surface area in determining the intestinal absorption and transport of drug molecules and has a negative correlation in that increased polar surface area results in decreased intestinal absorption and transport. The averaged polar solvent-accessible surface areas for the residues positioned to insert into the membrane (amino acids 8–11, 13, 20–25, 27 and the anthracycline) were calculated for each complex. These results show that in both models B and C, the anthracyline derivatives which do not induce translocation have increased polar surface areas over AD 198 that range from 4% higher for AD 288 in model B to over 50% for DOX in model B, Table 2. The differences are more consistent for the data derived from model C, where the smallest increase over AD 198 is 14% for AD 288 and 35% for DOX. The nonpolar surface areas for the same residues do not show the same correlation with the biological translocation data (data not shown).

The available biological data strongly implicate the 14-valerate substitution in the binding of AD 198 to C1 domains. Model B predicts a mode of binding dependent upon specific interactions between the 14-valerate alkyl chain with Leu 24 and the hydrogen bond between the C15 carbonyl oxygen and amide proton of Gly 23. In contrast, in model C neither the 14-valerate nor the N-benzyl moieties interact directly with the ΔC1b; however, in this model, both hydrophobic groups are positioned to interact with the membrane. Thus, model C reflects a role of these groups in establishing appropriate surface polarity and membrane interactions for optimal ligand binding to the C1b domain. Even though the 14-valerate and N-benzyl are not involved in specific hydrogen bonds, AD 198 shows significantly stronger hydrogen bonding in model C than does AD 288 or DOX. This is largely due to an increased strength of the hydrogen bond between Gly 23 and the C12

![Figure 6. Model C: hydrogen bonding.](image)

![Figure 7. (A,B) Translocation and translocation SAR.](image)
Models of AD 198 Binding to the PKC-δ C1b Domain

The present study compared three theoretical models to explain the role of the 14-acyl substituent in the interaction of AD 198 with PKC isoforms observed experimentally. Of these models, model C showed the greatest hydrogen bond energy between AD 198 and the C1b domain and also provided a trend in polar surface areas for AD 198, AD 288, and DOX that correlates with the experimentally measured ability of these compounds to induce translocation of PKC. Our previous studies demonstrated that AD 198 inhibits PKC in vitro. However, translocation is considered to be a hallmark of PKC activation by C1 ligands. Thus, the present experimental results appear to contradict the findings from our in vitro studies. This apparent contradiction is under further investigation in our laboratories.

The specific interaction of AD 198 with phorbol ester receptors provides an attractive explanation for the novel biological activity of this 14-acylate substituted anthracycline relative to the parental agents. Moreover, the interaction of AD 198 with highly conserved residues among typical C1 domains explains the interaction of AD 198 with diverse proteins, namely the classic novel PKC isoforms, as well as β2-chimaerin. Furthermore, based upon this finding, it is reasonable to expect AD 198 to interact with other C1 domain bearing proteins that have not yet been investigated. The current model provides the framework for the rational design of novel PKC directed anthracyclines with potentially improved pharmacological profiles. Further studies using site-directed mutants of the C1b domain and more extensive SAR studies will be required to further validate the binding mode of AD 198. These studies and the role of PKC isoforms and other phorbol ester receptors in the biological activity of AD 198 are currently a focus of our laboratories.

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References


Summary and Conclusions

The present study compared three theoretical models to explain the role of the 14-acyl substituent in the interaction of AD 198 with PKC isoforms observed experimentally. Of these models, model C showed the importance of the membrane environment in phorbol binding and for the activity of PKC is well-established.27,28 Presently, the inclusion of explicit lipid molecules is impeded by the lack of molecular details describing their interaction with the C1 domain and the computational time required to model lipid membranes. A model has been proposed for the binding of PKC to C1 ligands based upon biochemical data, the crystal complex of the C1b domain in the absence of lipid, and extensive SAR data.13,28,40 Briefly, ligand binding displaces water molecules from the groove between residues 6–13 and 21–27 of the C1 domain capping the polar binding pocket. The resulting contiguous hydrophobic surface inserts into the bilayer and anchors the protein to the membrane.

Based upon this model, assuming equivalent hydrogen-bonding interactions, ligands with reduced polar surface areas would be expected to have a higher affinity. Indeed, this relationship is observed for the phorbol esters with maximum affinity observed for the water-insoluble long chain fatty acid diesters in which the long chains can mask the polar surfaces in a hydrophobic environment.25 An analogous relationship may exist for the anthracyclines wherein hydrophobic anthracyclines such as AD 198 possess both sufficiently low polar surface areas in hydrophobic environments and appropriate heteroatom geometry for binding to C1 domains.

The interactions of DOX and AD 288 with lipid bilayers are markedly different than those observed for AD 198. AD 198 binds more tightly and the anthraquinone ring system is inserted more deeply within the membrane than observed with either DOX or AD 288.20 Explicit structural data are not available for AD 198 binding to lipid membranes. However, it is reasonable to speculate that both the valerate and benzyl groups allow AD 198 to embed into the lipid bilayer more readily than DOX or AD 288 by occupying an interfacial conformation similar to that shown in Figure 2 and masking polar regions that would impede such insertion. This interfacial orientation would be expected to facilitate the interaction of AD 198 with the C1 domain equally well in either model B or model C as demonstrated by the almost identical average polar surface areas shown in Table 2. Thus, the selective interaction of AD 198 with the C1b domain may be due to a decreased polar surface area relative to DOX and AD 288 and hydrogen-bonding interactions indirectly influenced by the 14-valerate substituent. Further studies using site-directed mutants of the C1b domain and more extensive SAR studies in concert with molecular modeling studies are underway to identify the correct binding mode.

Therefore the 14-valerate group exhibits its influence on hydrogen bond strength indirectly.

The importance of the membrane environment in phorbol binding and for the activity of PKC is well-established.27,28 Presently, the inclusion of explicit lipid molecules is impeded by the lack of molecular details describing their interaction with the C1 domain and the computational time required to model lipid membranes. A model has been proposed for the binding of PKC to C1 ligands based upon biochemical data, the crystal complex of the C1b domain in the absence of lipid, and extensive SAR data.13,28,40 Briefly, ligand binding displaces water molecules from the groove between residues 6–13 and 21–27 of the C1 domain capping the polar binding pocket. The resulting contiguous hydrophobic surface inserts into the bilayer and anchors the protein to the membrane.

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