

■ CHEMISTRY

Understanding Why the “Atypical” Protein Kinase C Isoforms Do Not Bind Phorbol Esters

Pu Y, Peach ML, Garfield SH, Wincovitch S, Marquez VE, and Blumberg PM. Effects on ligand interaction and membrane translocation of the positively charged arginine residues situated along the C1 domain binding cleft in the atypical protein kinase C isoforms. *J Biol Chem* 281: 33773–88, 2006.

The phorbol esters, classic tumor promoters, function as ultrapotent analogs of sn-1,2-diacylglycerol (DAG), the ubiquitous second messenger generated through the breakdown of phosphatidylinositol 4,5-bisphosphate. The recognition motif for the phorbol esters and DAG is the C1 domain, a 50 amino acid long zinc finger structure. Protein kinase C (PKC) constitutes the best known class of signaling proteins containing C1 domains, which represent hydrophobic switches. Phorbol ester/DAG inserts into a hydrophilic cleft in an otherwise hydrophobic surface on the C1 domain, providing a hydrophobic cap for this cleft and favoring insertion of the hydrophobic face of the C1 domain into the lipid bilayer. This insertion drives both the conformational change of PKC, causing its activation, as well as its membrane translocation, controlling its access to substrates.

PKC is a compelling therapeutic target both for cancer and a range of other conditions. Within the CCR, the Laboratories of Cancer Biology and Genetics and of Medicinal Chemistry bring together biological and chemical methodologies to understand ligand interactions with C1 domains and to exploit this understanding to develop therapeutic leads. This effort has led to the design of DAG lactones that have affinities approaching those of phorbol esters and that provide powerful chemical tools for probing biological questions. In contrast to the classical and novel PKCs, the “atypical” PKC isoforms zeta and iota have C1 domains that are phorbol ester/DAG unresponsive. We have now begun to apply the lessons from the C1 domains of the classical and novel PKC isoforms to understand the nature of these “unresponsive” C1 domains.

The C1 domains of PKC zeta and iota are distinguished by a high net positive charge, arising from four arginines rimming the binding cleft. To probe their potential role, we started with the phorbol ester-binding C1b domain of PKC delta (**Figure 1**) and mutated the corresponding residues to arginine singly or in combination. Individually, mutation caused only a modest loss of binding affinity, a modest decrease in membrane translocation of the

C1 domain in response to phorbol ester, and an enhanced requirement for anionic membrane phospholipids. Upon multiple mutations, binding and translocation were progressively abolished, yielding a C1 domain that behaved similarly to those of PKC zeta and iota.

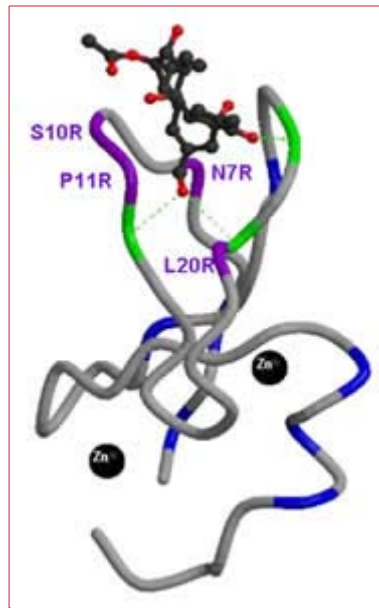


Figure 1. The structure of phorbol ester bound to the C1b domain of protein kinase C (PKC) delta is shown. The phorbol ester is portrayed as a ball-and-stick representation, with the carbon atoms in black and the oxygen atoms in red. The backbone of the zinc finger is indicated in grey. The four residues N7, S10, P11, and L20, which are present as arginines in PKC zeta and iota, are purple. The residues that hydrogen bond with the phorbol ester are in green (the hydrogen bonds are shown as green dotted lines), and the other positively charged residues are blue.

To determine whether other residues in the C1 domains of PKC zeta and iota, independent of these arginines, could abrogate phorbol ester responsiveness, we reciprocally mutated the four arginines in the C1 domains of PKC zeta and iota to the corresponding residues found in the C1b domain of PKC delta. The mutated C1 domains gained phorbol ester responsiveness, undergoing translocation in response to phorbol ester, albeit with potencies approximately 30-fold weaker than that of the C1b domain.

Computer modeling provides insight into the mechanism by which the arginine residues influence phorbol ester binding. The modeling predicts that the conformation of the binding cleft in the C1 domains of PKC zeta and iota is similar to that of the C1 domains that bind phorbol ester. However, the arginine residues along the rim of the binding cleft can swing into and occlude the binding pocket, thereby competing with the ligand for occupancy.

Approximately half of the 60 mammalian proteins with C1 domains have been described as phorbol ester unresponsive. Our results establish that the so-called phorbol ester unresponsive C1 domains fall into two categories, those retaining the intrinsic binding geometry of the cleft (e.g., PKC zeta and iota), and those that no longer contain such a binding site (e.g., Raf). An implication, supported by preliminary results, is that it may be possible to design ligands tailored to C1 domains such as that of PKC zeta and iota.

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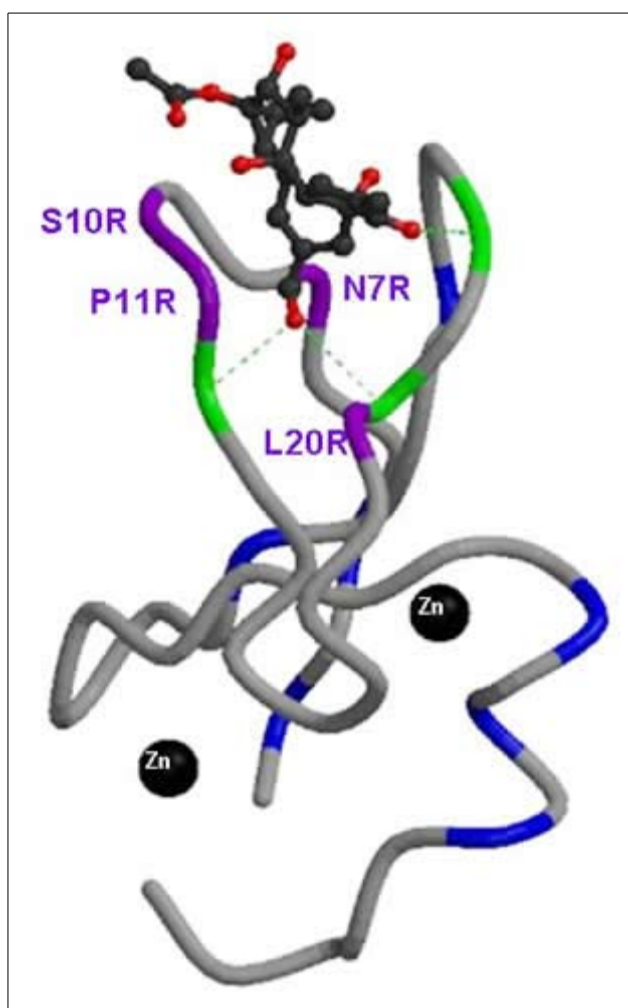


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