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Report:

Mammalian phosphoinositide-specific phospholipase C enzymes (PI-PLC) act as signal transducers that generate two second messengers, inositol-1,4,5-trisphosphate and diacylglycerol. Data collected at ESRF have been vital to our structural studies directed toward understanding the function of these enzymes in signal transduction. The structure of the δ1-isozyme revealed a multidomain protein incorporating modules shared by many signalling proteins (Essen *et al.*, 1996). Using data collected at BL4/ID13, we were able to obtain a set of structures that have helped us understand the catalytic mechanism of the *enzyme* and the means by which it interacts with phospholipid membranes. For proposal LS454 we collected 11 full data sets. The resolution limits ranged from 2.3 Å to 2.8 Å. To help decipher the catalytic mechanism, we collected data for complexes of substrate and transition state analogues for both the full-length enzyme and a deletion variant lacking the N-termnal PH domain (Essen *et al.*, 1997a). In an effort to define the role of the C-terminal C2 domain in binding phospholipid membranes in a calcium-dependent manner, we collected data sets for the enzyme in complexes with calcium, barium, and lanthanum (Essen *et al.*, 1997b). The structures derived from these data sets can be found in Protein Data Bank Entries lDJG, lDJH, 1DJI, lDJX, lDJY, and 1DJZ.

The substrate analogues used in our studies mimic the head group of the principal substrate involved in signal transduction, phosphatidyl-inosito14,5-bisphosphate (PIP2). The inositol phosphates we used include D-myo-inositol-1,4,5-trisphosphate, D-myo-inositol-2,4,5-trisphosphate, D-myo-inositol-4,5-bisphosphate and D,L-myo-inositol-2-methylene-1,2-cyclic-monophosphona~. The complexes exhibit an almost invariant mode of binding - each fitting edge-on into the active site and interacting with both the enzyme and the calcium cofactor at the bottom of the active site. Most of the active site residues do not undergo conformational changes upon binding either calcium or inositol phosphates. A network of hydrogen bonds and salt links between the active site and the bound inositol phosphate ensures that with the exception of the 6-OH group, all hydroxyls and phosphoryls are stereospecifically recognized. The structures am consistent with bidentate liganding of the catalytic calcium to the inositol phosphate cyclic intermediate and transition state

The complexes suggest explanations for substrate preference, pH optima, and ratio of cyclic to acyclic reaction products. The structures support general acid/base catalysis in a sequential mechanism involving a cyclic phosphate intermediate and rules out a parallel mechanism where acyclic and cyclic products are simultaneously generated.

We have determined the crystal structures of complexes PLC-81 with calcium, barium and lanthanum at 2.5 Å to 2.6 Å resolution. Binding of these metal ions is observed in the active site of the catalytic TIM-barrel and in the calcium binding region (CBR) of the C-terminal domain. The C-terminal domain is an eight-stranded antiparallel &sandwich of a type known as a C2 domain. Deletion variants lacking the C2 domain are catalytically inactive. By interacting with the EF-hand and catalytic domains, this domain is central to the interdoman packing in PLC-δ1. The domain has a structure very similar to the C2A domain from synaptotagmin I (SytI), however, the C2 domain of PLC-61 is a circularly permuted topological variant (P-variant) of the synaptotagmin I C2A domain (S-variant). Based upon sequence analysis, it appears that both S-variant and P-variant topologies are present among C2 domains in other proteins. Like SytI C2A, PLC-61 binds calcium and calcium analogues. Metal ion binding sites involve residues in the β1/β2, β3/β4, and β5/β6 loops. The analogous calcium binding in SytI C2A brings about binding to anionic phospholipid headgroups. It may be that the C2 domain of PLC-61 has a similar role. Multiple adjacent binding sites in the PLC-61 C2 domain were observed for calcium and the other metal/enzyme complexes. The maximum number of binding sites observed was for the calcium analogue lanthanum. This complex shows an array-like binding of three lanthanum ions.

Several domains appear to simultaneously interact with the membrane and enable the enzyme to carry out hydrolysis of membrane-resident substrates: the N-tetminal PH domain tethers the enzyme the membrane by interacting with phosphoinositide headgroups; the C2 domain might fix the orientation of the enzyme on the membrane by binding to anionic phospholipid headgroups in a calcium-dependent manner. The enzyme oriented at the membrane interface would then be able to carry out multiple rounds of catalysis without dissociating from the membrane.

Essen, L.-O., Perisic, O., Cheung, R., Katan, M. & Williams, R. L. (1996). Crystal structure of a mammalian phosphoinositide-specific phospholipase Cδ. *Nature 380,595-602.*

Essen, L.-O., Perisic, O., Katan, M., Wu, Y., Roberts, M. F. & Williams, R. L. (1997a). Structural mapping of the catalytic mechanism for a mammalian phosphoinositide-specific phospholipase C. Biochemistry 36(7), 1704-1718.

Essen, L.-O., Perisic, O., Lynch, D. E., Katan, M. & Williams, R. L. (1997b). A ternary metal binding site in the C2 domain of phosphoinositide-specific phospholipase C-61. ***Biochemistry* in press.**