Activated PLCγ Breaking Loose

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In this issue of Structure, Bunney and colleagues use a combination of NMR, SAXS, crystallography, ITC, and biochemical methods to elucidate, in molecular detail, the sequence of events causing receptor-mediated activation of phospholipase C-γ1 by protein tyrosine phosphorylation.

Inositol phospholipid-specific phospholipases C (PLCs) catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdInsP2) to produce inositol 1,4,5-trisphosphate (InsP3) and diacylglycerol (DAG). The enzymes thus produce three immediate intracellular messages: increased InsP3 (and, consequently, a rise in intracellular Ca2+), increased DAG (causing enhanced activity of protein kinases C), and decreased PtdInsP2. The local concentration of the latter in the plasma membrane and, possibly, in other cellular membranes regulates the activities and/or subcellular distribution of many regulatory or structural proteins, including enzymes, ion channels and transporters, transcription factors, scaffolding proteins, and cytoskeletal proteins. The thirteen mammalian PLC isoforms are divided into six subfamilies, designated β, γ, δ, ε, ζ, and η (Kadur and Ross, 2012). The four members of the PLCβ subfamily are activated by heterotrimeric G protein Gαq and Gβγ subunits as well as, in the case of PLCβ2, by the Rho GTPases Rac and Cdc42. The two members of the PLCγ family, γ1 and γ2, are activated by receptor and nonreceptor protein-tyrosine kinases. PLCγ2, but not PLCγ1, is also activated by Rac. In addition, alternative mechanisms exist to regulate the activity of the two PLCγ isoforms. The regulation of the other PLC isoforms (δ-η) is less clear, although they too may be activated by increased intracellular Ca2+ (δ-η), heterotrimeric G protein subunits (ε, η), and small GTP-binding proteins (δ, ε). Intriguingly, some of the cellular functions of PLCγ isoforms appear to be independent of their catalytic activity (e.g., Swiercz et al., 2009).

The molecular mechanisms of activation of the PLC isoforms in intact cells, in particular by regulatory proteins and by tyrosine phosphorylation, have long been obscure and have only recently been elucidated by structural studies. Thus, analysis of the three-dimensional structure of a truncated form of PLCβ2 revealed that the active site of the enzyme is occluded by a small segment of the region separating the two halves (X and Y) of the catalytic triose phosphate isomerase barrel (Hicks et al., 2008). Deletion of this segment constitutively activates PLCβ2 without ablating its capacity to be further stimulated by Gαq, Gβγ, or Rac. Similar regulation occurs in other PLC members (δ, ε). This has led to the proposal of a general mechanism of interfacial PLC isozone activation. According to this model, the various protein activators would anchor and orient the PLCs at substrate membranes to release autoinhibition by the XY linker and to promote access of PtdInsP2 to the lipase active site. However, several lines of evidence suggest that other mechanisms of PLC activation and autoinhibition exist, even for PLCβ (Lyon et al., 2011), but also for PLCζ (Nomikos et al., 2012) and the PLCγ isoforms, which do not contain negative charges in their XY linkers at the high density critical in other PLCs for interfacial activation and are not activated by deletion of a segment corresponding to the autoinhibitory XY linker segment of PLCβ (unpublished data).

The sequence linking X and Y in PLCγ isoforms is unique and encodes a modular tandem comprising, in this order, the N-terminal half of a split PH domain (nSH2), an N-terminal SH2 domain (nSH2), a C-terminal SH2 domain (cSH2), an SH3 domain, and the C-terminal half of the spPH domain (cSPH). The whole tandem is also referred to as specific array (SA). Previous results on the activation of PLCγ1 by tyrosine phosphorylation have shown that nSH2 is important for recognition of phosphorylated tyrosine residues on activated cell surface tyrosine kinase receptors, whereas cSH2 interacts, in an intramolecular fashion, with the major phosphorylated tyrosine of PLCγ1, Y783, located immediately downstream of cSH2 (Poulin et al., 2005; Gresset et al., 2010).

The work by Bunney et al. (2012) in this issue of Structure reports the structures of the whole PLCγ1, SA (as well as single, isolated domains and combinations thereof), as determined by 2D 15N,1H-HQC. NMR spectroscopy and the structure of the tandem nSH2-cSH2 pair solved by X-ray crystallography. Cross peaks detected in isolated cSH2 were not observed (in contrast to those corresponding to the other SA constituents) unless SH2 domain ligand peptides were present, suggesting that the latter may destabilize transient interactions between cSH2 and the rest of SA. Such interactions may occur between the cSH2 and both nSH2 and SH3. The tandem nSH2-cSH2 pair displays relatively low intramolecular flexibility with respect to each other. MONSA and XPLOR-NIH analysis of SAXS data revealed that the spPH and cSH2 domains form the central lobe of the SA molecular volume, which is sandwiched between the nSH2 and SH3. The crystal structure of nSH2-cSH2 (including the cSH2-SH3 linker containing phosphorylated Y783) showed that the side chains of the latter and three adjacent residues make multiple interactions with the conserved pY binding site of cSH2 without changing the overall structure of the domain. Comparison of the binding of the Y783-phosphorylated and the nonphosphorylated nSH2-cSH2 domain to activated, Y766-phosphorylated FGFR1 by ITC revealed that wild-type nSH2-cSH2 interacted with the receptor with high (Kd ~5 nM) and low affinities.
(K_D ~ 81 nM), whereas nSH2-cSH2-pY783 interacted with the receptor via only a single binding site with an intermediate affinity (K_D ~ 44 nM). Collectively, these results and other evidence presented in the paper suggest that phosphorylation of Y783 of PLCγ1 weakens the interaction of the enzyme with the activated receptor. To investigate the connection between Y783 phosphorylation and increased PLCγ1 activity, the interaction in trans of 15N-labeled nSH2, cSH2, and spPH with the PLCγ1 core lacking SA was examined by NMR titration. Most intriguingly, only cSH2 showed changes in its NMR spectrum, and these changes were completely reversed in the presence of the phosphorylated cSH2-SH3 linker peptide containing pY783. These findings and other evidence imply that cSH2 is, in fact, the only SA constituent that interacts with the PLCγ1 core containing its catalytic domain and that this interaction can be released following Y783 phosphorylation and intramolecular association with the cSH2 domain. Finally, the authors used biochemical experiments to demonstrate that the association of wild-type, full-length PLCγ1 with activated FGFR-1 is greatly decreased following PLCγ1 phosphorylation and that phosphorylated, isolated PLCγ1 retains high activity without the requirement of sustained binding to activated FGFR-1.

The findings reported in this work have several important implications. First, they strongly suggest a model of PLCγ1 activation by tyrosine kinase growth factor receptors, consisting of an initial and transient strong interaction between the receptor and PLCγ1, mediated by nSH2, followed by phosphorylation of PLCγ1-cSH2-mediated release of catalytically active PLCγ1 from the receptor, and, possibly, processive phosphorylation of many PLCγ1 molecules by a single activated receptor. Depending on the relative lifetimes of the activated states of the individual proteins, this could lead to signal amplification at a very early step of signal transduction, similar to that observed, in many cases, for the activation of heterotrimeric G proteins by their receptors (e.g., Arshavsky et al., 2002). Second, the structural alterations observed in the PLCγ1 SA during receptor-mediated activation may help to understand the molecular mechanisms involved in activation of PLCγ2 by chemically induced or spontaneously occurring mutations in the mouse Plcg2 and the human PLCG2 genes, respectively, and giving rise to specific autoimmune diseases in mice and humans, such as cold-induced urticaria and immune dysregulation (see Zhou et al., 2012 for references). Intriguingly, several of these mutations map to cSH2 or the interface between csPH and cSH2, as determined in the study reported in this issue.

It seems clear that this work leaves certain aspects open and raises new questions. For example, it is unclear to what extent the findings reported here for PLCγ1 can be extrapolated to PLCγ2, which plays important, specific roles in, e.g., B cells, monocytes/macrophages, mast cells, and platelets. Analysis in intact B cells suggests that phosphorylation of more than one site may be required for full enzyme activation (e.g., Watanabe et al., 2001). Second, the functional roles of the other PLCγ1 SA constituents, such as spPH and SH3, and phosphorylation(s) of PLCγ1 at sites potentially functionally relevant in other cellular contexts and for other types of stimuli, such as Y775 and Y1253, remain to be integrated into the model suggested here. Finally, many of the results presented in this paper were (and had to be) done in transfected cells or cell-free preparations. It is clear that experiments in native cells will be required to determine the validity of the concepts derived in this very elegant study for the behavior of PLCγ1 in natural plasma membranes and more complex cellular environments.

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REFERENCES


