Structure-function relationship of broad-range phospholipase C from Listeria monocytogenes

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Broad-range phospholipase C (PC-PLC) is a metalloenzyme and a crucial virulence factor of *Listeria monocytogenes* (*Lm*). Crucial step in *Lm* pathophysiology is the escape from the lipid encased phagosome after internalization. Disintegration of the phagosome membrane is facilitated by three key bacterial proteins: pore-forming cholesterol dependent cytolysin listeriolysin O (LLO), and two listerial phospholipases C: PC-PLC and PI-PLC. PC-PLC can hydrolyse a variety of lipid substrates, including those having phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine headgroups as well as sphingomyelin. We recombinantly expressed the enzyme and purified PC-PLC to high concentrations, enabling us crystallisation and determination of its crystal structure at 2.0 Å resolution. With structure solved, we searched for mutants retaining wild-type protein fold, while exhibiting lower enzymatic activity. The prime candidate was N-terminal Trp1 residue, which coordinates one of the Zn^{2+} ions and anchors the N-terminus in the hydrophobic core of the protein. We prepared mutants where W1 was replaced by A, E, F, or K or was deleted (dW1S2). While all mutated proteins retained wild-type fold, they had mostly reduced enzymatic activity: wild-type W1 ~ W1F >> W1A > W1E ~ W1K ~ dW1S2. To decipher the interplay between LLO and PC-PLC, we pre-incubated POPC/SM/cholesterol lipid vesicles with PC-PLC. PC-PLC caused significant increase in LLO binding to liposomes and LLO induced vesicle leakage, while PC-PLC on its own did not cause any permeabilization. Preincubation with less active PC-PLC mutants resulted in reduced LLO binding and lower vesicle leakage. Those findings suggest that activity of PC-PLC may increase the availability of membrane cholesterol. Further structure-based functional studies of PC-PLC with LLO are in progress, aiming towards a better understanding of the mechanism and interplay between these two toxins of *Lm*.