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Determining the Structure of Phospholipase C Epsilon

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ABSTRACT

The phospholipase C (PLC) epsilon subfamily of PLC enzymes are found at highest concentration within the cardiovascular system. Improper functioning of the enzyme, whether due to overstimulation or changes in expression, has far-reaching effects within the human body Stunted heart valve development and cardiac hypertrophy and are two such examples. The mechanisms by which PLC epsilon activity is regulated in these processes remain unknown, as does the physical structure of the enzyme. In this study, we seek to determine the structure of a PLC epsilon fragment that retains enzymatic activity and is amenable to crystallization. Mutagenesis of PLC epsilon cDNA was performed to remove two peripheral flexible domains of the enzyme that are dispensable for catalysis. This DNA was subsequently used to generate a baculovirus and infect insect cells for protein expression. The protein of interest was harvested, purified, and subjected to crystallization screening. By removing domains known to be flexible, we are more likely to obtain well-ordered crystals, amenable for determining a high-resolution structure. The structure of this domain variant will next be determined via X-ray diffraction. This structure is a key starting point for unravelling the complete structure of PLC epsilon and better understanding its mechanism of action.

KEYWORDS

Crystallography, enzyme structure, cardiovascular