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Structural Characterization of the DEP Domains of P-Rex1

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ABSTRACT

P-Rex1 is a guanine nucleotide exchange factor for Rho-GTPases, which is indirectly involved in the regulation of cell migration and proliferation. It contains a tandem DH/PH domain archetypal of the Dbl family of GEFs, two DEP and two PDZ domains, and a C-terminal end with weak homology to inositol polyphosphate 4-phosphatase. P-Rex1 is regulated by both intra-domain interactions and interactions with other proteins such as G-protein beta gamma, PKA and phosphatidylinositol (3,4,5)-trisphosphate. Upregulation of P-Rex1 has been found in multiple human cancers, making it a potential target for anti-cancer drug therapies. Therefore, structural characterization of P-Rex1 is critical. Currently, only the structures of the DH/PH tandem and PDZ1 domains of P-Rex1 have been determined. The goal of this project is to determine the structures of the DEP1 and DEP2 domains using X-Ray crystallography. P-Rex1-DEP1 (409-499 aa) protein was expressed in *Escherichia coli* and purified using affinity and size exclusion chromatography. The purified protein was then concentrated and used to set various crystallization screens. Small, well defined needles were observed and showed UV absorption, indicating that they consist of protein, and thus represent promising leads for a future structure determination. Optimization is in progress to grow bigger crystals or establish new conditions. Attempts are still being made to purify P-Rex1-DEP2 (500-602 aa), which thus far shows tendencies to aggregate.

KEYWORDS

P-Rex1, DEP Domain, Structure, X-Ray Crystallography