Crystallization of TIPRL, a Novel Regulator of Type 2A Phosphatases

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Type 2A phosphatases are part of the PPP subfamily that is formed by PP2A, PP4 and PP6, and participate in a variety of cellular processes including transcription, translation, regulation of the cell cycle and apoptosis. Each of these phosphatases has its own regulatory subunits, but they also share two common regulators, TIPRL and a4. In a previous work we investigated the ternary complex formed by PP2Ac, a4 and TIPRL. The function of TIPRL is still poorly understood and it does not presents sequence similarity to any other proteins family. The objective of this study is to crystallize TIPRL and determine its three dimensional structure. The 3D TIPRL structure may reveal its mechanism of interaction with the catalytic subunit PP2Ac. Initial attempts to crystallize full length TIPRL were unsuccessful. Prediction of secondary structure indicated that 12 residues of the N-terminal and 18 residues of the C-terminal region of TIPRL are not structured which may affect crystallization. Deletion mutants were produced lacking either the 12 N-terminal (TIPRL ?N), the 18 C-terminal unstructured residues (TIPRL ?C*)* or lacking both of them (TIPRL?NC). Constructs lacking the 18 C-terminal residues were expressed as inclusion bodies indicating that an intact C-terminal is important for TIPRL accurate folding. Soluble TIPRL ?N was abundantly expressed in *E. coli*. Purification of TIPRL ?N was carried out using IMAC and size exclusion chromatography. Six initial TIPRL ?N crystallization screens were performed at 12 mg/mL. Crystalline structures were observed after 15 days. Crystal optimization is in progress and best crystals were obtained with TIPRL at 2 mg/mL using 100 mM NaAc pH 5.5; 35% PEG and 200 mM Li₂SO₄ as precipitant.

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