Expression, Purification, and Crystallization of the AtRLI2 Protein (RNase L Inhibitor), a suppressor of RNA Silencing from *Arabidopsis thaliana*.

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The "RNase L inhibitor" (RLI) is a protein highly conserved in eukaryotes and Archaea and was first identified in humans, where it acts as a regulator of 2-5A synthase/RNase L pathways. Two new functions have been described for RLI, the first related to ribosomal proteins binding and initiation of translation, and the other to the control of RNA silencing. Both the RNA silencing as well as the 2-5A synthase/RNase L pathways respond to double stranded RNA molecules, playing key roles in the regulation of gene expression and resistance to viruses. Aiming future structure and function studies, isolated domains of AtRLI2 (NBS and Fe-S) were cloned into the bacterial expression vector pET-28a, expressed in *Escherichia coli BL21 DE3 pRIL codonplus* and purified. Unfortunately, the NBS domain was insoluble and cold not be purified. However, the FeS domain was soluble, and after its purification was used for various purposes: i) analysis of biophysical properties ii) initial test of crystallization iii) production of antibodies. The Fe-S domain was purified by Nickel Chelate Affinity Chromatography showing income greater than 80 mg/liter of induction. The secondary structure of the purified protein was evaluated by Circular Dichroism (CD) indicating that it presents defined secondary structure. The polydispersity and hydrodynamic radius of the protein in solution were evaluated by Dynamic Light Scattering (DLS) indicating that the protein is in the form of a tetramer. Among the conditions tested, the most appropriate to minimize the polydispersity was buffer 20 mM Tris-HCl, pH 7.0 with 100 mM NaCl a 10ºC. In these conditions, 8.9% of polydispersity was obtained. Finally, to obtain antibodies, mice (High IV-A) were immunized. Analysis of antibody titration made by ELISA showed that the produced antibodies recognize the peptide with a title of 1/2560.

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