

Abstract

Background: FIP-Lrh is a fungal immunomodulatory protein from Tiger Milk Mushroom with immunomodulatory and cytotoxic properties. Its biological properties can be further elucidated with the availability of a crystal structure. However, crystal structures from only four FIPs have been reported so far. As such, previous study at UCSI and MGVI on rFIP-Lrh crystallisation under two conditions gave hexagonal, irregular, and microcrystal structures, which warranted further optimisation.

Objectives: To produce recombinant rFIP-Lrh in *E. coli* BL21 expression system and to optimise conditions for crystallisation of the rFIP-Lrh.

Methodology: *E. coli* FIP-Lrh/pET-28a(+) transformants cultured in LB broth were induced for expression with IPTG. Total cell lysates containing soluble rFIP-Lrh were purified using Ni-NTA affinity chromatography, dialysed, and subjected to further purification using size exclusion chromatography. Protein crystallisation was performed using three screening kits: Crystal Screen™ 1 and 2, PEG/ION Screen™ 1 and 2, and NeXtal Tubes Classics Suite. Further optimisation was performed to obtain a refined crystal structure of rFIP-Lrh.

Results: Soluble rFIP-Lrh (14.9 kDa) was successfully produced with a yield of 100 mg/L of expression culture. After a few rounds of screenings, two optimised crystallisation conditions successfully generated orthorhombic crystals: (i) 0.2M potassium sodium tartrate tetrahydrate, 0.1M sodium citrate tribasic dihydrate pH 5.6, 2.0M ammonium sulphate and (ii) 0.2M K/Na tartrate, 0.1M tri-sodium citrate pH 5.6, 2.0M ammonium sulphate.

Conclusion: The project's objectives were achieved. To protect the crystals during storage and subsequent X-ray diffraction analysis, suitable cryoprotectants (such as glycerol and polyethylene glycol) will be used in the future. The X-ray diffraction data obtained can be used to generate the biological 3D structure of FIP-Lrh for further functional analysis.

Keywords: Tiger Milk Mushroom, Fungal Immunomodulatory Protein, FIP-Lrh, Protein Crystallisation