

Crystallization And Preliminary Structural Studies Of *Schizolobium parahyba* Chymotrypsin Inhibitor In Complex With Chymotrypsin

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Schizolobium parahyba chymotrypsin inhibitor (SPCI) is a small protein with 180 amino-acid residues able to inhibit chymotrypsin at a 1:1 molar ratio by forming a stable complex. The binary complex of the SPCI with chymotrypsin was purified by size-exclusion chromatography. The crystallization was achieved by the sitting-drop vapour-diffusion method. An automatic system for crystallization using Matrix Maker and Honeybee robots was used to set up 96-well crystallization plates. Initial crystallization trials were performed using different precipitant types from commercial screens. The best crystals were grown in drops using a solution consisting of 100 mM MES–NaOH pH 5.5, 20%(w/v) PEG 6000, 200 mM LiCl as precipitant and 200 mM nondetergent sulfobetaine molecular weight 201 Da (NDSB-201) as an additive. X-ray data were collected to 2.8 Å resolution from a single crystal under cryogenic conditions. The crystal belongs to space group $P2_12_12_1$, with unit-cell parameters $a = 45.28$, $b = 64.57$, $c = 169.23$ Å, and the R_{merge} is 0.122 for 11 254 unique reflections. A molecular-replacement solution was found using the preliminary crystal structure of SPCI and the structure of chymotrypsin (PDB code 4cha) independently as search models. At present, refinement is being performed and new crystallization attempts are being carried out in order to improve crystal quality and resolution.

Keywords: SPCI-chymotrypsin complex, crystallization, X-ray diffraction.

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