

Mexicain, from the Crystal to the Structure: A Sixty Years Journey

Luis A. González-Ramírez^a, Carmen Oliver-Salvador^b, Jose A. Gavira^a, Manuel Soriano-García^c, Juan M. García-Ruiz^a, ^a*Laboratorio de Estudios Cristalográficos. IACT, CSIC-Universidad de Granada. Granada, Spain.* ^b*UPIBI, IPN. México, D.F. México.* ^c*Instituto de Química, UNAM, México, D.F. México.* E-mail: lagonzal@ugr.es

Mexicain is a cysteine protease from the tropical plant *Pileus Mexicanus* (now, also called *Jacaratia mexicana*) first described by Castañeda and co-workers in 1942 [1]. Previous crystallization trials of mexicain were reported but unfortunately the quality of the crystals was not good enough for accurate X-ray analysis [2, 3].

In this work we present the strategy to find crystallization conditions that produce crystals of the complex protein-inhibitor that are suitable for x-ray diffraction studies. We will present purification protocols and biochemical characterization of mexicain as well as the crystallization of mexicain bound to the inhibitor by vapor diffusion and counter-diffusion techniques. Crystals were cryo-protected with glycerol to a final concentration of 20%. Frozen crystals were analyzed using an X-ray rotating anode source and they diffracted to a resolution of 1.94 Å. The crystal belongs to the monoclinic space group $P2_1$ with unit cell parameters $a = 57.36$; $b = 90.45$; $c = 80.39$; and $\beta = 92.64$. The asymmetric unit contains four molecules of mexicain with a corresponding crystal volume per protein mass (V_m) of 2.24 and a solvent content of 45% by volume.

[1] Castañeda-Agulló M., Gavarrón F.F., Balcazar M.R., *Science*, 1942, **96**, 365. [2] Castañeda-Agulló M., Hernández A., Loaeza F., Salazar V., *J. Biol. Chem.*, 1945, **159**, 751. [3] Oliver-Salvador M.C., Moreno A., Soriano-García M., *Ciencia (México)*, 2000, **51**, 12.

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