The 1.31 Å Resolution Crystal Structure of VapC21 from *Mycobacterium tuberculosis*

Jardim, P.[1]; Santos, I.C.S.[1]; Valadares, N.F.[1]

[1]Laboratório de Biofisica Molecular, Departamento de Biologia Celular, Universidade de Brasília, Brasília, Brazil.

Introduction and objectives: The main molecular machineries that drive cells into a persistent state are the toxin-antitoxin systems, that participate in the general stress response, including hypoxia and nutritional stress, and whose *loci* are widespread in bacteria. The *Mycobacterium tuberculosis* H37Rv genome presents 88 toxin-antitoxin *loci*, 45 of them corresponding to the vapBC family, a type II TA system that function as an operon expressing a toxin (VapC) with cytotoxic activity and an antitoxin (VapB) that binds and inhibit the toxin. In these complex self-regulated systems the antitoxin is capable of binding specific DNA sequences to regulate the transcription of the operon. Our aim is to express, purify, crystallize and perform the biophysical characterization of these proteins.

Materials and methods: Competent *E. coli* BL21 (DE3) pLysE cells were transformed using expression vectors pET24a+ coding for the toxin VapC21. After several expression tests, the protein was purified using a Ni^{++} affinity column and a Superdex 75 column. SDS-PAGE was used to monitor the process. DLS was used to monitor the protein dispersity. The pure protein was concentrated at 4 °C in an Amicon Ultra-15. Automated crystallization assays were performed using a Mosquito HTS nanolitre dispenser and commercial crystallization screening kits. The initial conditions were developed in manual crystallization assays using 1.5 µL of VapC21 at the concentration of 6 mg/mL and an equal volume of the reservoir solution in a siting drop plate. The X-ray data was collected at the LNLS, and was processed, resolved and refined by using the programs XDS, Phaser, Coot and Phenix.

Results and conclusions: The VapC21 protein was expressed, purified and crystallized. The structure at 1.31 Å resolution reveals important novel details in the active site, dimerization interface and in the putative antitoxin interaction sites.

Key words: Toxin, Antitoxin, Tuberculosis, Crystallography

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