Title: Structural biology of enzymes involved in the (S)-4-amino-2- hydroxybutyrate (AHBA) biosynthesis from the aminoglycoside butirosin

Authors: Laura A. Rivas; Fernanda C. Rodrigues de Paiva; Fanglu Huang; Peter Leadlay; Marcio V.B. Dias

Institution: Instituto de Ciências Biomedicas ICB II. (Av. Professor Lineu Prestes, 1374, CEP 05508-000, São Paulo, Brazil); Department of Biochemistry, University of Cambridge; Department of Chemistry, University of Warwick

Natural products obtained from secondary metabolism of bacteria, plants and fungi represent an important source of antimicrobial agents for the treatment of diverse illness, such as infections. One of these agents is the aminoglycosides, molecules that have precursors from the glycolytic pathway in bacteria, as butirosin, which is produced by *Bacillus circulans*. One of its particularities is the presence of an (S)-4-amino-2-hydroxybutyrate (AHBA) attached to the 2-deoxystreptamine (2-DOS) aglycone ring. The presence of AHBA in butirosin makes this antibiotic less susceptible to bacterial resistance mechanisms through aminoglycosides modifying enzymes. However, the biosynthesis of the AHBA group in the butirosin pathway is not sufficiently understood, although it is known the participation of six enzymes in its biosynthesis whose structures have not been extensively studied. Herein, we aim to perform a structural analysis of three of these enzymes: BtrJ in charge of the addition of molecules of L-glutamate; BtrK which participates catalyzing a decarboxylation reaction of glutamate; and BtrH which participates of the final steps transferring the AHBAs group from an acyl carrier protein to a ribostamycin molecule. Genes *btrJ, btrK* e *btrH* were cloned in pET28a plasmid vector. BL21(DE3) or Rosetta competent cells were used for transformation and expression of the protein. Ni^{2+}NTA affinity and size-exclusion chromatography were performed to purify the proteins and subsequent protein crystallography experiments were performed by sitting drop and hanging drop. Crystal X-ray diffraction data collection was performed at Synchrotron Light Source DESy (PETRA III, Germany) or LNLS (Campinas). Data for BtrK crystals have been obtained at a resolution of about 1,4 Å in the space group P21212. The crystallographic refinement was carried out by the CCP4i software package. BtrK is a two-domain protein, one of them with a TIM barrel folding which comprises of the PLP active site and possibly, the region that interacts with other substrates. Comparing to other decarboxylases, the active site is conserved and includes the Schiff base between the catalytic lysine (K49) and the PLP coenzyme. The general electrostatic surface is slightly negative while the PLP pocket is positively charged. This pocket is still characterized to be large and shallow. BtrJ and BtrH enzymes experiments are still in progress.

Keywords: butirosin, AHBA, decarboxylase, structural biology
Funding: FAPESP, CAPES, CPNq