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Structural Characterization of the Mutant E197D, an Epoxide Hydrolase of Lasalocid Biosynthesis

Wilson, C.[1, 2]; Huang, F.[3]; Santos, J. C.[2]; Leadlay, P. L.[3]; Dias, M. V. B.[1, 2]

[1]Instituto de Ciências Biomédicas, ICB-USP, SP; [2]Programa de Pós Graduação em Microbiologia,IBILCE-Unesp, SP, Brazil; [3] Department of Biochemistry, University of Cambridge-UK; carol87.wilson@gmail.com

Abstract: The polyethers antibiotics play several biological roles such as antifungal, anticancer and neuroprotective activities. Based on these characteristics, these compounds have become a target for the bioengineering of natural products, where the manipulation of their biosynthesis offers new opportunities in drug development [1]. The polycyclic polyethers are derived from the epoxidation of polyenes followed by a linear cascade of opening of cycle epoxides. This process involved two classes of enzymes, a monooxygenase containing flavin and an epoxide hydrolase [2], that in general, occur by favorable reactions exo cyclization. However, the synthesis of some of these natural products occurs through kinetically unfavorable reactions, termed anti-Baldwin cyclization reactions, where the endo type cyclization occurs, such as in the biosynthesis of Lasalocid [3-5] by epoxide hydrolase. During the biosynthesis of this compound, LasB (the epoxide hydrolase) takes the polyketide intermediate in bisepoxide tetrahydropyran-tetrahydrofuranyl. The structure of LasB in complex with an analog of substrate has been elucidated, and the C-terminal domain of the enzyme is responsible for the unusual cyclization process [6]. The objectives were to start a crystallography study of the several mutants of LasB to clarify the mechanism of catalysis of this enzyme and provide information about the specificity by substrate of the two domains of this protein. More specifically this work had to show the structure of the Mutant E197D of LasB and obtain insights about the effect of shorter side chain of the this amino acid in the active site of this enzyme. The LasB with the mutation E197D was constructed by site directed mutagenesis using the wide type *lasB* gene cloned in pET28a(+) and its expression was carried out using BL21(DE3). E197D LasB mutant was purified using affinity and gel filtration columns. The crystal was obtained using hanging drop vapour diffusion method. Diffraction data was collected at the PETRA-III beamline, at Germany and processed with XDS and SCALA. The programs PHASER, COOT, PHENIX, MOLPROBITY and PyMol were used to the structure refinement and analysis of final models. Crystals of E197D LasB mutant was obtained in a condition with high concentration of sodium formate and they diffracted at a resolution of 1.6 Å and belongs to the space group P6322. The structure was solved by molecular replacement and contains a monomer in the asymmetric unit. Although we have added 5 mM of valpromide, the analysis of the active site did not show any clear electronic density for this compound.

References:

[1] UEBERBACHER, B. T.; HALL, M.; FABER, K. Electrophilic and nucleophilic enzymatic cascade reactions in biosynthesis **Nat. Prod Rep.**, n. 29, p. 337−350, 2012.

[2] MINAMI, A.; OGURI, H.; WATANABE, K.; OIKAWA, H. Biosynthetic machinery of ionophore polyether lasalocid: enzymatic construction of polyether skeleton. **J. Am. Chem. Soc.**, n.134 (17), p. 7246–7249, 2012.

[3] BALDWIN, J. E. J. Rules for Ring Closures. **Chem. Soc.**, **Chem. Commun.**, n.18, p.734−736, 1976.

[4] BALDWIN, J. E.; KRUSE, L. I. J. Rules for ring closure. Stereoelectronic control in the endocyclic alkylation of ketone enolates. **Chem. Soc.**, **Chem. Commun.**, n. 7, p. 233−235, 1977.

[5] MORTEN, C. J. et al. The Development of Endo-Selective Epoxide-Opening Cascades in Water. **Chem. Soc. Rev.**, n. 38, p. 3175−3192, 2009.

[6] FONG, T. et al. Epoxide Hydrolase−Lasalocid A Structure Provides Mechanistic Insight into Polyether Natural Product Biosynthesis. **J. Am. Chem. Soc.**, n. 137, p.86−89, 2015.