5th BCNP Brazilian Conference on Natural Products
XXXI RESEM
Atibaia – SP – Brazil
ISBN: 978-85-66836-10-3
Oct. 26–29th 2015

TRIDIMENSIONAL STRUCTURE OF A METHILTRANSFERASE FROM GENTAMICIN GENE CLUSTER

Bury, P.S.[1], Huang, F.[2], Leadlay, P.[2], Dias, M. V. B.[1]

1 Universidade de São Paulo, Instituto de Ciências Biomédicas, Departamento de Microbiologia, SP, Brazil; 2 Department of Biochemistry– University of Cambridge-England; priscilabury@usp.br

Abstract: Gentamicin aminoglycosides are broad spectrum antibiotics from *Micromonospora echinospora*. However, its clinical use is limited due to toxic effects, such as nephrotoxicity and ototoxicity [2-5]. To bypass this issue, one strategy consists in manipulating the biosynthetic pathways of the aminoglycosides in order to generate new suitable derivatives of these molecules. To do so, we are seeking to characterize structurally enzymes responsible for aminoglycoside biosynthesis. Thus, we have focused on GenN, which is an enzyme involved in the gentamicin biosynthesis. GenN is a SAM methyltransferase with a hypothetical function that catalyzes the addition of a methyl group at the garosamine sugar of gentamicin [3-6]. The objective of this work was to determine the first tridimensional structure of GenN. **Material and methods.** *genN* was cloned into pET28a and superexpressed in BL21(DE3). The purification was performed using immobilized metal affinity chromatography and size exclusion chromatography. To crystallize this enzyme, it was incubated with the co-factor adenosylmethionine prior to the crystallization screen assays. Crystals were obtained and they were manually reproduced and optimized. Diffraction experiments were done at PETRA III, Germany, and a complete data set was obtained. The data processing was performed using the program XDS[4]. The structure was determined by SAD using iodine derivative using the CCP4 program [1]. **Results.** GenN crystallized in the space group P212121 with one molecule in the asymmetric unit. The crystals diffracted up to 2.0 Å of resolution and the structure refinement is in progress. **Conclusion.** The structure of GenN was obtained for the first time and with the conclusion of this structure, we will obtain key insights about the mechanism of the methylation catalysed by this enzyme. This information also could be very useful to generate new aminoglycoside derivatives *in vitro* through enzymatic engineering.

References

[1] CCP4 Acta Crystallogr D Biol Crystallogr. Available from:< http://www.ncbi.nlm.nih.gov/pubmed/15299374> Cited: 27 Jun. 2014.

[2] Davies, J. In the beginning there was streptomycin. Aminoglycoside antibiotics: from chemical biology to drug discovery, New Jersey, p. 1-12, 2007.

[3] Hong, W. R., Ge, M., Zeng, Z. H., Zhu, l., Luo, M. Y., Shao L., Chen, D. J.. Molecular cloning and sequence analysis of the sisomicin biosynthetic gene cluster from Mycromonospora inyoensis. Biotechnol Lett, Fuzhou, v. 31. p. 449-455, 2009.

[4] KABSCH, W. XDS. Biological Crystallography, v. 66, p. 125-132, 2010

[5] MOULDS, F. W., JEYASINGHAM, M. S. Gentamicin: a great way to start. Australian Prescriber, v. 33, n. 5, p. 134, 2010.

[6] PARK, S. R., Park, J. W., Ban, Y. H., Sohngb, J. K., Yoon, Y. J.. 2-deoxystreptamine-containing aminoglycoside antibiotics: recent advances in the characterization and manipulation of their biosynthetic pathways. Nat Prod Rep, v. 30, p. 11-20, 2013.