

MS06-2-3 Trapping a novel intermediate of vitamin B6 biosynthesis in PLP synthase, using in crystallo spectroscopy

#MS06-2-3

M. Rodrigues¹, N. Giri², A. Royant³, Y. Zhang⁴, R. Bolton¹, G. Evans⁵, S. Ealick⁴, T. Begley², I. Tews¹

¹University of Southampton - Southampton (United Kingdom), ²Texas A&M University - College Station (United States), ³Institut de Biologie Structurale - Grenoble (France), ⁴Cornell University - Ithaca (United States), ⁵Diamond Light Source - Didcot (United Kingdom)

Abstract

The major pathway of natural vitamin B6 biosynthesis is the ribose-5-phosphate dependent pathway. PLP is an active form of vitamin B6 that is synthesised by PLP synthase, an enzyme complex consisting of up to 24 protein subunits of the two proteins Pdx1 and Pdx2. Pentose and triose carbohydrates and ammonia, derived from glutamine hydrolysis catalysed by Pdx2, are the substrates of vitamin B6 biosynthesis. The Pdx1 synthase subunit catalyses a complex sequence of chemical reactions through formation of covalent enzyme-intermediate complexes, using lysine amino acid residues in an unusual double Schiff base mechanism.

We have characterised the I333 intermediate, named after its characteristic absorbance, using a lysine-arginine exchange variant. Using in crystallo UV-vis absorption spectroscopy, mass spectrometry analyses and structure determination we propose I333 as an on-pathway intermediate. The structure of the enzyme intermediate complex rationalises stereoselective deprotonation and subsequent substrate assisted phosphate elimination, which are central to our understanding of PLP biosynthesis.

References

Rodrigues MJ, Windeisen V, Zhang Y, Guédez G, Weber S, Strohmeier M, Hanes JW, Royant A, Evans G, Sinning I, Ealick SE, Begley TP, Tews I. Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis. *Nat Chem Biol.* 13 (2017), 290-294.

Rodrigues MJ, Giri N, Royant A, Zhang Y, Bolton R, Evans G, Ealick SE, Begley T, Tews I. Trapping and structural characterisation of a covalent intermediate in vitamin B6 biosynthesis catalysed by the Pdx1 PLP synthase. *RSC Chem Biol.* 3 (2021), 227-230.