Poster Presentation

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Structural characterisation of a polyphosphate kinase

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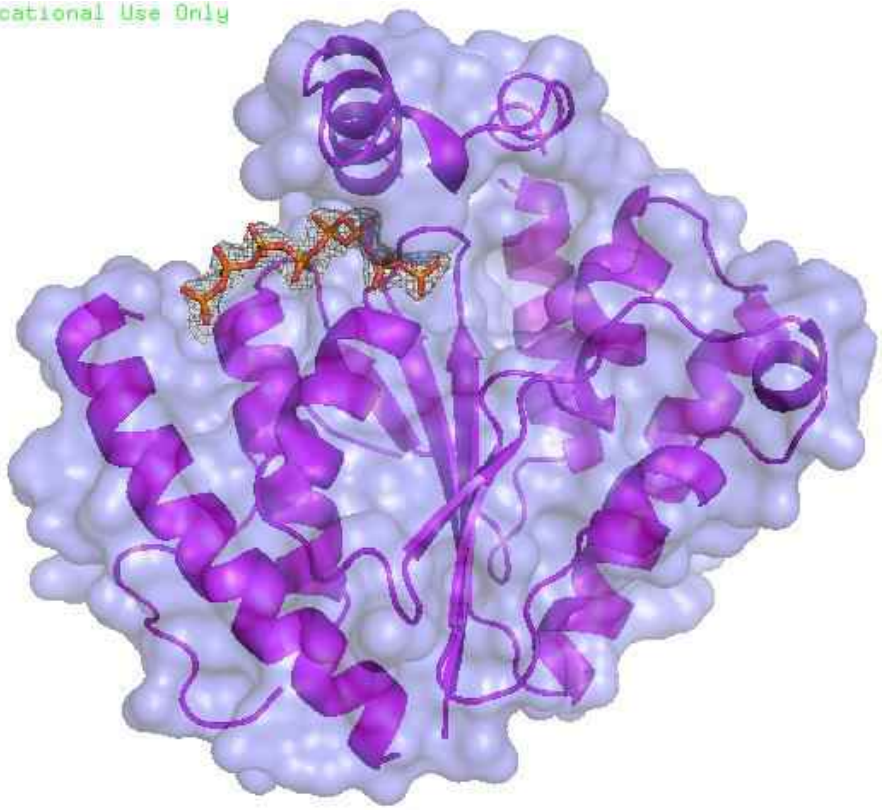
When deprived of nutrients bacteria undergo what is referred to as the 'stringent response'. During the stringent response, the cell induces the expression of genes to cope with stress and starvation and diverts resources away from cell growth and division. This involves altering the cellular levels of the signalling molecules: ppGpp, pppGpp and inorganic polyphosphate (polyP)[1]. This is controlled by four enzymes; Polyphosphate kinase (Ppk), Exopolyphosphatase (Ppx), RelA and SpoT. Therefore, modulation of these enzymes is an attractive method for targeting pathogenic bacteria such as Francisella tularensis (F. tularensis), the causative agent of tularemia. FtPpk transfers phosphate from polyP to ADP to generate ATP, a reaction that is fully reversible. The importance of FtPpk in infection has been demonstrated in knockout mutants which resulted in defective growth of F. tularensis in macrophages[2]. Mutagenesis in other pathogenic bacteria has yielded attenuated mutants, suggesting an important role for Ppk in a broad spectrum of bacterial species[3]. To maximise our understanding of FtPpk, our aim was to obtain co-crystals of the enzyme and substrates. Isothermal Titration Calorimetry (ITC) was used to measure the binding of polyP and ADP to FtPpk as independent substrates. FtPpk binds ADP very weakly or not at all in the absence of polyP. FtPpk binds polyP in an exothermic reaction with a relatively high affinity (0.385 µM) in the absence of ADP. Co-crystals of FtPpk with polyP and ADP have been obtained and optimised to diffract to 2.0 Å, identifying a potential binding site for polyP. A non-hydrolysable analogue of ATP has been chemically synthesised to allow co-crystallisation experiments.

[1] *Jain, V., M. Kumar, and D. Chatterji, ppGpp: stringent response and survival. J Microbiol, 2006. 44(1): p. 1-10.,* ***[2]*** *Richards, M.I., S.L. Michell, and P.C. Oyston, An intracellularly inducible gene involved in virulence and polyphosphate production in Francisella. J Med Microbiol, 2008. 57(Pt 10),* ***[3]*** *Gangaiah, D., et al., Polyphosphate kinase 2: a novel determinant of stress responses and pathogenesis. PLoS One, 2010. 5(8): p. e12142.*



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