[MS5-P47] High resolution analysis of the pyrrole polymerising enzyme porphobilinogen deaminase from *Bacillus megaterium*.

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The enzyme porphobilinogen deaminase (PBGD) (hydroxymethylbilane synthase) catalyses a key early step of the haem and chlorophyll biosynthesis pathways in which four molecules of the monopyrrole, porphobilinogen, are condensed to form a linear tetrapyrrole. The active site possesses an unusual dipyrromethane cofactor which is covalently attached to the enzyme at an invariant cysteine residue. The cofactor acts as a primer which is extended during the reaction by the sequential addition of the four substrate pyrrole molecules. The Gram-positive bacterium *Bacillus megaterium* is of great industrial interest since it has many applications in the biotechnological production of numerous substances, including the tetrapyrrole vitamin B_{12}. Until very recently, structural data have only been available for PBGDs from *Escherichia coli*, humans and *Arabidopsis*. However, expression in *E. coli* of the PBGD from *B. megaterium* using the expression vector pET14b (kind gift of Prof. M. Warren, Kent) has allowed the enzyme from this organism to be nickel-affinity purified and crystallised. Curiously, the cells and purified protein have an intense pink colouration, but the crystals (which were obtained in 0.2 M magnesium acetate, 0.1 M sodium cacodylate pH 6.5 and 20 % PEG 8000) are yellow. X-ray analysis at DLS beamline I03 yielded diffraction data to 1.46 Å resolution with a Pilatus detector. Data processing using iMOSFLM indicated that the crystals belong to the space group $P2_12_12_1$ and have unit cell dimensions of a = 53.3 Å, b = 65.8 Å, c = 97.2 Å with one molecule per asymmetric unit and a solvent content of 42 %. The data have an overall *R*-merge of 6.1 %, 100 % completeness and greater than 6-fold redundancy. Molecular replacement using MOLREP in the CCP4 suite yielded an excellent solution with the *E. coli* enzyme (which has 48 % sequence identity) as the search model. Refinement and interpretation of the *B. megaterium* PBGD structure is in progress using the near-atomic resolution data, with current values of the *R*-factor and *R*-free being 14.5 and 19.0 %, respectively. The electron density map provides excellent evidence for alternative forms of the dipyrromethane cofactor, most probably the dipyrromethene and dipyrromethanone forms which may account for the pink and yellow colour of the protein in different oxidation states. This is in contrast to our recent high resolution work on the *Arabidopsis* enzyme in which evidence for only the fully oxidised dipyrromethenone form was obtained.