Phasing phosphoribosyl isomerase (PriA) from *Streptomyces coelicolor* using SeMet-MAD at BM14, ESRF

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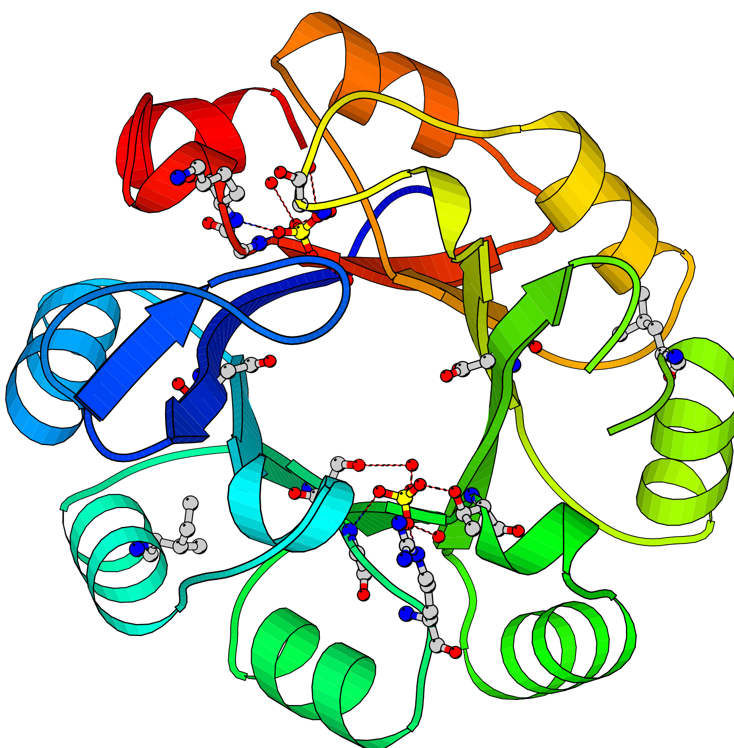
Phosphoribosyl isomerase (PriA) is a recently discovered enzyme with a dual function involved in both histidine and tryptophan biosynthesis in the high (G + C)-content Gram-positive bacteria *Streptomyces coelicolor* and *Mycobacterium tuberculosis* (Barona-Gómez & Hodgson, 2003). The discovery of the dual functionality of PriA arose from the observation that the genome of *S. coelicolor* and *M. tuberculosis*, as well as several other actinomycetes, contains no apparent *trpF* gene. It has previously been shown *in vivo* that both HisA and TrpF activity are present in the gene product of *priA* using deletion mutants and complementation studies (Barona-Gómez & Hodgson, 2003).

We have heterologously expressed and purified *S. coelicolor* Pria in *Escherichia coli* as a N-terminal His-tag fusion. The purified recombinant enzyme was crystallized using the hanging drop method in 1.50 *M* ammonium sulphate and 100 m*M* sodium citrate pH 4.8 (Wright et al.; 2004). Due to the relatively low sequence identity (27%) to related TIM-barrel enzymes, molecular replacement failed to obtain the correct solution. Despite the presence of three cysteine residues, heavy atom derivatives could not been obtained due to the low pH of the crystallization mother liquor. An earlier S-SAD experiment was also unsuccessful (4 S (3Cys and 1Met) atoms/240 a.a. residues) due to the relatively poor quality of the crystals.

We have engineered a second Met into the protein and subsequently obtained Se-Met crystals (2Se atoms/240 a.a. residues). A 3-wavelength MAD dataset was collected to 2Å resolution, 360° each, providing 6-7 fold anomalous redundancy. Location of the two anomalous Se scatterers, and initial phasing and density modification were carried out using SHELXD and SHELXE. The contrast and connectivity figures of merit for the correct heavy-atom enantiomer ($P3_121$) were 0.52 and 0.94, respectively, as opposed to 0.29 and 0.87, respectively for the wrong hand. About 70% of the structure was autotraced by RESOLVE using the phases obtained from SOLVE. It was interesting to see that SAD phases obtained using only the peak data were comparable to those generated by the 3-wavelegth MAD data. The structure has been refined at 1.8Å resolution and showed, as anticipated the characteristic $(\beta\alpha)_8$-barrel fold. There are two phosphate binding sites at the molecule.



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References:

Barona-Gómez, F. & Hodgson, D. (2003). *EMBO reports* **4**, 296-300.
Wright, H., Barona-Gómez, F., Hodgson, D.A. & Fülöp, V. (2004). *Acta Cryst.* **D60**, 534-536.