Poster Presentation

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Case studies of sulfur SAD phasing with LigM from Sphingobium sp. SYK-6

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Sphingobium sp. SYK-6 grows on a lignin-related biphenyl compound as the sole carbon and energy source and was initially isolated from a pond waste liquor from a kraft pulp mill. In SYK-6, 5-CH3-H4folate is synthesized from aromatic compounds such as a vanillate by a O-demethylase, LigM. The 5-CH3-H4folate is then converted to 5,10-CH2-H4folate, which is utilized for syntheses of DNA, repair DNA, and methylate DNA as well as to act as a cofactor in certain biological reactions, by another enzyme, MetF. In other bacterial speceis, 5,10-CH2-H4folate is directly synthesized by T- and H-proteins that are enzymes in Glycine Cleavage System. It is considered that SYK-6 has evolved to acquire this unique pathway for the 5,10-CH2-H4folate production, in order to survive in extreme environmental condition. To elucidate the molecular mechanisim of this pathway, we have carried out the structural analysis of LigM. LigM was purified by using IMPACT system provided from NEB, which use intein and affinity chitin-binding tag. After crystallization screening, a reservoir solution of 0.2 M Mg acetate, 0.1 M Acetate buffer pH 4.6, and 20 %(w/v) PEG8000 gave a needle crystals with approximate dimensions of 0.3×0.1×0.01 mm3. A diffraction data set was collected with 1.1 Å wavelength at BL1-A in the Photon Factory. However, phasing trials via molecular replacement (using a model with 19% sequence identity) failed. Because LigM is a 53 kDa protein and contains fourteen sulfur atoms, LigM is an interesting candidate for SAD phasing with sulfur (S-SAD). Diffraction data sets of LigM crystals were collected with 1.9 and 2.7 Å wavelengths, reaching a maximum resolution of 2.3Å. Preliminary results are promising for solving the phase problem via S-SAD. This study is also of methodological interest as the phasing capability of two different wavelengths can be compared. A thorough analysis of the diffraction data is in progress.

Keywords: S-SAD, Protein Crystallography, Enzyme