Preliminary X-ray analysis of urate oxidase from *Bacillus sp.*

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Urate oxidase (uricase, EC 1.7.3.3; UOX) is an essential enzyme responsible for the conversion of uric acid to allantoin, catalyzing the oxidative opening of the purine ring during the purine degradation pathway. Enzymatic analysis of uric acid in blood with UOX is commonly used for the diagnosis of gout.

The chemical mechanism of UOX reaction has not been established, and is particularly interesting in light of the fact that the enzyme neither contains nor requires any cofactor. Recently, the structure of eucaryotic UOX has been determined (N. Colloc'h *et al.*, *Nature Struct. Biol.*, **4**, 947, (1997)). However, the question still remains why UOX catalytic reaction readily occurs at room temperature, although the direct reaction of O_2 in a triplet ground state with a singlet substrate to give singlet products is a spin-forbidden process.

Here we report the preliminary X-ray diffraction analysis of UOX from *Bacillus sp.* TB-90 having better thermostability and higher activity across the wide range of pH 6-9 than the eucaryotic one.

The purified protein was concentrated to 10 mg ml^{-1} by ultrafiltration for crystallization. Crystals of the UOX complexed with 8-azaxanthine were grown by the hanging-drop vapor-diffusion technique. Protein solution (5 μl) was mixed with an equal volume of reservoir solution and the mixture was equilibrated against 1 ml of reservoir solution at 293K. The UOX crystals were measured at 100 K after soaking the crystal dialyzed against a cryo-protectant solution containing 20 % PEG 400. X-ray diffraction data were collected using a Mar CCD detector and synchrotron radiation on beam line BL41XU. A total of 180° of data were measured using the oscillation method. Individual frames consisted of a 1° oscillation angle measured for 10 sec at a crystal-to-detector distance of 130 mm. Intensity data were processed using MOSFML.

UOX crystals grew to maximum dimensions of 0.4 × 0.2 × 0.02 mm within two weeks. The crystal was of good quality, diffracting to 2.3 Å resolution or better. Data reduction was performed and the space group and unit cell parameters were determined using the MOSFML and the CCP4 program suite (Collaborative Computational Project, Number 4, 1994). The space group was monoclinic $P2_12_12$, with unit-cell parameters a =78.8, b =133.5, c =144.4 Å, $\alpha=\beta=\gamma=90°$. Assuming one UOX subunit (M.W. 38,000) per asymmetric unit, the Matthews coefficient is calculated to be 2.51 Å^{3}Da^{-1}, corresponding to a solvent content 50.6%. The data are 96.4% complete to 2.2 Å resolution, having a total number of 435,579 reflections containing 71,652 unique reflections. The completeness in the 2.32-2.20 Å shell is 90.0%. R_{merge} for the entire data set collected on a single crystal is 9.2% (30.1% for the outer shell). Further data analysis is now underway.

X-ray crystallography of the complex of SeqA, a negative regulator of replication, and a hemi-methylated DNA

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Escherichia coli chromosomal DNA has approximately 1900 GATC sequences, which are methylated by Dam methyltransferase. In the DNA replication step, until the synthesizing dauter strand is methylated by Dam methyltransferase, the chromosomal DNA is kept in the hemimethylated state. *Escherichia coli* SeqA protein binds to the hemimethylated sequence in the *ori* region to restrict one cycle of replication per one cycle of cell division. To elucidate the mechanism how SeqA strictly recognizes the hemimethylated DNA, we crystallized the complex of SeqA and 10 bp or 16 bp DNA including hemimethylated adenosine. Crystals of the complex with 16 bp hemimethylated DNA belong to the mnoclinic spacegroup $C2$ (a=84.19 Å, b =67.98 Å, c = 88.21 Å, β =110.6°), and diffract X-ray up to 2.65 Å resolution. The Rmerge and the completeness were 0.038 (0.114) and 91.1% (57.1%), respectively (the values at the outer shell are shown in parentheless). However, the Wilson plot implies that the crystals are twined, and we could not determined the phase by Se-MAD phasing. On the other hand, crystals of the complex with 10 bp DNA belong to the hexagonal spacegroup $P622$ (a=b =152.96 Å, c = 119.1 Å), and diffracts X-ray beyond 3.2 Å resolution. By MAD method using SeMet derivative, we determined the phase and building the atomic model is now in progress.