2001A0526-UML-np BL41XU

A Phasing Trial applied on the High Resolution X-ray Data from a FMN-binding protein by a Single Anomalous Dispersion (SAD) method with Sulfur atoms.

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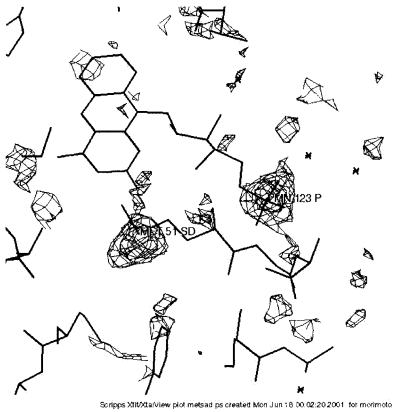
A protein crystallography is the most powerful technique to determine a protein 3-D structure. The Fourier density map presents us many important aspects of proteins. It is , however, necessary to retrieve phase information to calculate the Fourier density map. The multiple-isomorphous replacement method (MIR) is the best and most useful way to determine phases of reciprocal X-ray data. However, it needs at least two heavy atom derivatives, and three high quality data sets. If sulfur atoms in protein molecules are available for phase determination, it is good to solve protein structure. B.C.Wang shows the phase problem is overcome by use of the single anomalous dispersion of sulfur atoms and its density modification with high resolution data from a native protein molecule (1985, Method in Enzymology, vol.115). According to this way, we tried phasing of X-ray data from FMN-binding protein obtained at the BL41XU undulator beam line , SPring-8.

A FMN-binding protein has the cell dimensions a= 36.622, b=83.755, c=40.412Å, β=93.493°, space group P2$_1$, and two molecules in the asymmetric unit and it gives an atomic resolution diffraction data. A MAR-CCD165 detector was set on the position from crystal with 120mm and off-setting of 60mm from direct beam position. A wavelength was 1.7Å, and exposure time was 30sec/frame. A total of 392 frames was collected.

Results of data processing with keeping the Bijvoet pair are good and the coincident R-factor up to 1.92Å is 3.2%. Anomalous signal (f'') is sufficient for finding sulfur atoms in the anomalous Fourier density map. The map shows also phosphor density of a FMN-group. A phasing trial from these peaks is now in progress.



2001A0531-NL -np BL41XU

Crystal structure analysis of RecJ protein by MAD method

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RecJ is a 5' to 3' exonuclease specific for single-stranded DNA. RecJ is involved in three DNA repair systems; recombinational repair, methyl-directed mismatch repair, and base excision repair. RecJ has five characteristic motifs, and the homologues containing these motifs expand from prokaryotes to higher eukaryotes. However, the role of these motifs is unclear, and the structures of RecJ and its homologues are not known. For functional and structural studies of RecJ protein, we constructed the truncated *recJ* gene that encodes the core domain and expressed it in soluble form. This domain contains all motifs and has full exonuclease activity.

We previously crystallized RecJ protein from *Thermus thermophilus* HB8 by hanging drop vapor diffusion method. By the same method, Se-Met RecJ was successively crystallized. Crystal of Se-Met RecJ is cubic, space group P2$_1$3 with unit cell parameters of a = b = c = 141.6 Å. The MAD data sets were collected at 100 K using an MARCCD detector in the beam line BL41XU. The Se absorption edge was determined by XAFS measurement using Se-Met RecJ protein. Diffraction images were recorded with 0.5° oscillation. All data sets were processed and scaled using HKL2000. Difference patterson map has located four Se atoms in RecJ protein.

Table 1. Data collections

	peak	edge	remote
Wavelength (Å)	0.9793	0.9795	0.9722
Resolution (Å)	20.0-3.2	20.0-3.3	20.0-3.3
Observed reflections	361587	356555	394380
Unique reflections	29761	27332	27181
Completeness	99.6 (99.9)	99.6 (100)	99.5 (99.8)
R_{sym} (%)	7.2 (29.1)	6.2 (25.9)	7.5 (32.6)

Values in parentheses are for the outermost resolution shell