2002A0042-NL1-np BL40B2

Collection of MAD dataset of Bacteriophage lambda CII Protein using Seleno-met derivative

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XAFS experiment was conducted on a frozen crystal to determine the optimum wavelengths (peak of absorption, edge and a remote wavelength) to be used for collecting data: 0.9796 (peak), 0.9798 (edge) and 0.9837 Å (low energy remote). After a few attempts we found a seleno-met crystal which diffracted to about 3 Å. We set up for collecting 180° data sets at the three wavelengths with 50 secs exposure per frame. As the quality of diffraction deteriorated the remote dataset could not be collected. We looked for a new crystal, but could get one which diffracted to only about 4 Å, for which complete data (at three wavelengths) were recorded.

Computation carried out in the home lab

The data were processed using MOSFLM and scaled using SCALA. Apparently, the 2nd crystal we used for collecting the MAD data sets had a slightly different cell dimension along the c axis; as such these data have not been used so far. The two data sets (at peak and inflection wavelengths) from the 1st crystal processed very well to about 3.2 Å (Table 1).

As the dataset corresponding to the remote wavelength was not available, we used a normal native data (collected on a rotating anode) as the remote dataset and ran SOLVE to search for the selenium sites. The cII protein has five Met residues in the poly-peptide chain, so that the tetramric molecule would have a total of 20 seleno-Met sites in the asymmetric unit of the crystal. SOLVE picked up 12 sites; but the correlation of anomalous differences at different wavelengths was not good involving the remote dataset as expected. So it was decided to try SAD (at the peak data) and then improve the phases using density modification. 16 consistent Se sites were obtained using both the programs SOLVE and CNS and the match between the predicted Patterson using these sites and the anomalous difference Patterson is reasonably good. We are now in the process of improving the phases and fitting the model into the map.

Table 1. Data statistics for the peak wavelength (values in parentheses are for the highest shell)

Wavelength (Å)	0.9796
Unit cell parameters (Å): *a, b, c*	63.381,107.588, 119.562
No. observed reflections	30,420
No. unique reflections	7,034
Resolution (Å)	32.28-3.2 (3.36-3.20)
R_{sym} (%)	6.7(31.2)
I/σ	7.4(2.3)
Completeness (%)	99.9
Redundancy	4.3(4.5)

2002A0071 BL40B2

Magnetic field effect of sample preparation of purple membranes.

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Purple membrane is two dimensional crystal of bacteriorhodopsin (BR), which is a proton transport membrane protein of *Halobacterium Salinarium*. X-ray diffraction experiments using purple membranes have played important roles in the study of BR. We measured x-ray diffraction of piled purple membranes (pH7, 298K) on a piece of mylar sheet. Diffraction peasks up to 3 Å were observed clearly. We can also distinguished small diffraction peak from purple membrane up to about 2.5 Å. Bragg peaks up to 5 Å are enough to separate to analyze the BR structure by the method we have employed. But at much higher angle, Bragg peaks broaden and separate badly. Therefore we tried to improve the quality of data by making a sample in magnetic field.

To make oriented purple membranes sample, we ordinary dry purple membrane suspension on a quartz sheet. In this experiment, we did this procedure in magnetic field (~2T) . If orientation of the sample is good, intensity ratios of each diffraction of tilted sample to X-ray will be differed from the untilted sample. Purple membrane suspension was pH7. We attached the sample to goniometer and measured X-ray diffraction by 2-degree step. The sample was kept at 298K.

By tilting the sample step by step, diffractions that were vertical to the rotation axis decreased gradually and could not be observed over 20 degree. Moreover diffraction intensities decreased without changing of the intensity ratios of each diffraction. Therefore, magnetic field does not effect on sample quality.

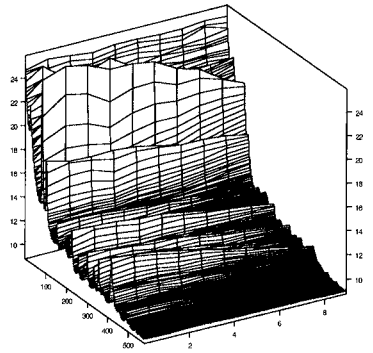

Fig.1 Tilt angle dependence of X-ray diffraction of purple membrane. Purple membranes are tilted by 2 dgree to X-ray axis.