X-ray crystal structure analysis of ribosomal protein S7 by MAD

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INTRODUCTION

We had tried to obtain good crystals of ribosomal protein S7 for data collection. Unfortunately, we could not get good crystals until our beamtime, and we couldn't collect good diffraction data of ribosomal protein S7 during our beamtime. Therefore, we decided to collect diffraction data from other crystals to check performance of the beamline. Two proteins were used for this purpose. One is 1-amino-cyclopropane-1-carboxylate (ACC) deaminase from yeast and the other is MIF-related protein 8 (MRP8) from human blood. The former crystal is sensible to X-ray radiation and data collection at cryo-temperature is indispensable, and diffraction is poor (~4Å resolution) using the laboratory equipment. The latter crystal is relatively small (~0.15^3mm^3) and only ~6Å resolution data can be obtained using the laboratory equipment.

RESULTS

ACC deaminase

ACC deaminase crystal co-crystallized with PHMBS (*p*-hydroxymercuri benzene-sulfonate) was used. We tried to measure fluorescence spectrum from PHMBS co-crystal with help of Dr. Kawano in SPring-8 to obtain maximum signal of mercury atom in a crystal, but it failed. Then we choose 0.98Å X-ray to obtain large anomalous signal of mercury atom. Statistics of data reduction is summarized in the table 1 and an example of Bijvoet difference Patterson map is shown in the figure 1.

MRP8

We tried to collect diffraction data of MRP8 without freezing. It diffracted higher than 2.0Å at the beginning of data collection, however, it decayed very quickly after a few images. It was impossible to obtain full set of diffraction data.

DISCUSSIONS

BL41XU has good performance for high resolution data collection from small crystal, and energy resolution seems to be good enough for MIR-OAS/MAD measurement. However, because of high intensity, crystal freezing might be indispensable for good data collection.

Because of high background X-ray, we could not observe absorption edge of mercury from a sample crystal. XAFS measurement system should be improved to reduce background noise. Solar slit or detector which has energy resolution, such as SSD or photo diode, may improve signal-to-noise ratio of fluorescence.

Table 1. Statistics of data reduction of ACC deaminase

Space group	$P3_221$
Cell dimension (Å)	$a = b = 80.1$, $c = 247.2$
Resolution limit (Å)	40-2.3
Wavelength (Å)	0.9800
Rmerge (%) [§]	4.5 (30.3)
No. of observed reflections	129,904
No. of unique reflections	37,871
Multiplicity	3.4 (3.3)
Rejected outliers [†]	249
Mean $<I/\sigma(I)>$	8.5 (2.5)
Completeness (%)	91.0 (76.9)

The data were processed with DENZO and scaled with CCP4 package. Values in parentheses are for the highest resolution shell (2.42-2.30Å).
[§] $R\text{merge} = \Sigma\Sigma_i |I(h)-I(h)_i|/\Sigma\Sigma_i I(h)$, where $I(h)$ is the mean intensity after rejections.
[†] Reflections with intensities differing more than $4.0\sigma(I)$ from the weighted mean were rejected.

Figure 1. Bijvoet Patterson map of PHMBS-yACCD