Bio-Crystallography

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The "Bio-Crystallography" beamline at the station BL41XU was open for the use from October 1997. The "X-ray Structural Biology" subgroup (SG) has been supporting the construction and operation of this beamline and several SG members have already got an opportunity to use this beamline and obtained many valuable diffraction data.

1. Beamline

The light source of Bio-Crystallography beamline (BL41XU) is an in-vacuum-type undulator of a magnetic periodicity of 3.2 cm and a total length of 4.5 m. The undulator gap can be changed from 8 mm to 50 mm. The energy range of fundamental emission is from 6 keV to 18 keV, and the corresponding energy range of third harmonics is from 18 keV to 54 keV. Using this light source, highly brilliant X-rays are available from 9 keV to 38 keV for the routine crystal structure analysis of biological macromolecules with the multiple isomorphous replacement technique with optimized anomalous scattering (MIR-OAS).

The front end elements such as graphite filters, apertures, an absorber, X-Y slits and a Be window work well at the storage ring current of 20 mA, all of which have possibilities to be exposed to the tremendous heat load from the light source.

The rotated-inclined double crystal monochromator (RIDCM) is the first element on which the high heat load is deposited. The water cooling pin-post structure in the first inclined crystal removes the total power up to 2 kW and the power density up to 100 kW/mrad2 at the storage ring current of 20 mA. The rotation mechanism of RIDCM is for amaintaining the foot print length of incident undulator light constant on the monochromator surface, but it has not yet been tested.

Although the RIDCM works enough as a cooling device, the performance as an X-ray optical element is not so high. The distortion of Bragg net plane, introduced at the pin-post construction, diverges reflected X-rays in horizontal direction twice compared with the ideal value. The energy resolution of the RIDCM has not yet been tested.

Since the undulator of the Bio-Crystallography beamline is installed at a high-b section of the storage ring, the source profile has a spatial anisotropy. In order to achieve a quasi-isotropic beam profile at sample position, the Bio-Crystallography beamline utilizes two X-ray mirrors in the KB configuration. That is, the mirror bending parameters can independently be controlled. This is favorable to realize a focused beam smaller than 0.2 mm (FWHM) in vertical and horizontal directions (Fig. 1). Using the KB mirror system, high brilliance beam at wavelength of 1.0Åwas obtained over a flux of 10^{12} phs/sec at a beam size of 0.1 mm.

An on-line imaging plate (IP) diffractometer is installed in an experimental station. The automatic exposures on Weissenberg photography have already been achieved, but the on-line readout mechanism for the exposed IPs is still under construction. All IPs were digitized so far with a Rigaku IP reader of dram scanner type. Several test data sets were collected by the beamline constructing group using hen egg white lysozyme (HEWL)

crystals at room temperature and trypsin crystals at cryo-temperature. The data acquisitions were carried out at 1.3Å resolution with a newly developed software system; AUTO (by Dr. Higashi at the Rigaku Co.). The Rmerge values were reduced to 5% at 2.0Å resolution for all data sets.

When users come to use the Bio-Crystallography beamline, the X-ray damage on the sample crystals will be a serious problem. Except the tough crystals such as HEWL, the cryo-cooling was indispensable for almost of all crystals in the use of this beamline.

(Nobuo Kamiya)

2. Experimental Results in Beamline

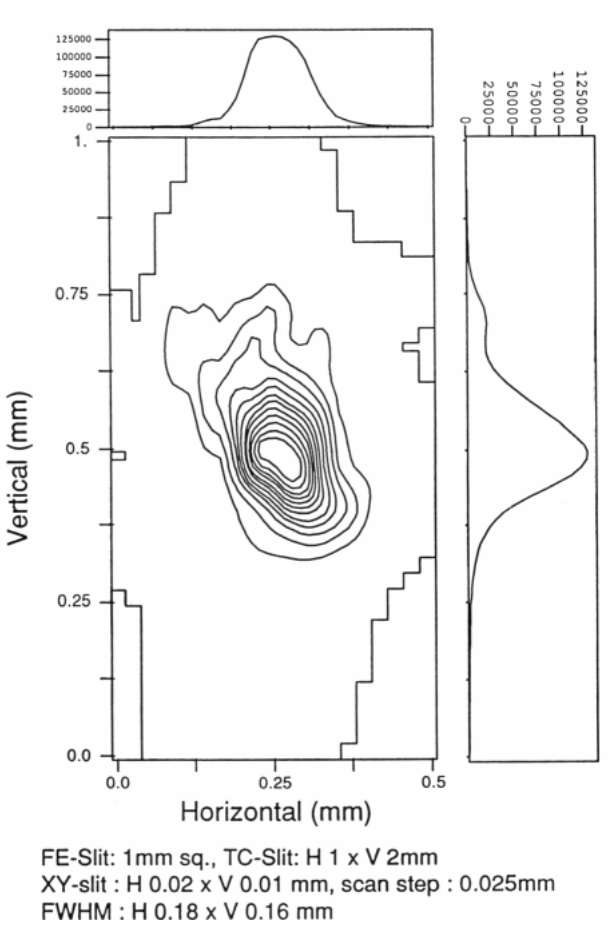

Fig. 1. Two-Dimensional Scan of Focal Spot at BL41XU

2.1. Cytochrome c oxidase

Cytochrome c oxidase from bovine heart is a typical respiratory membrane protein complex which catalyzes dioxygen reduction coupling with proton translocation across a membrane. Although we have solved the crystal structure of the enzyme at 2.3Å resolution, there remain unclear water structures which may have important roles in proton transfer within the enzyme. In order to clarify the water structures the crystal was frozen at 100 K. Cell dimensions of the frozen crystal used are a=187.3Å, b=207.2Åand c=178.6Å It diffracted to 2.1Å resolution. The diffraction image taken by 0.5 deg oscillation showed very sharp spots with a half-value width of about 150 mm, which is about one third of those taken at BL6A of the PF.

(Tomitake Tsukihara and Shinya Yoshikawa)

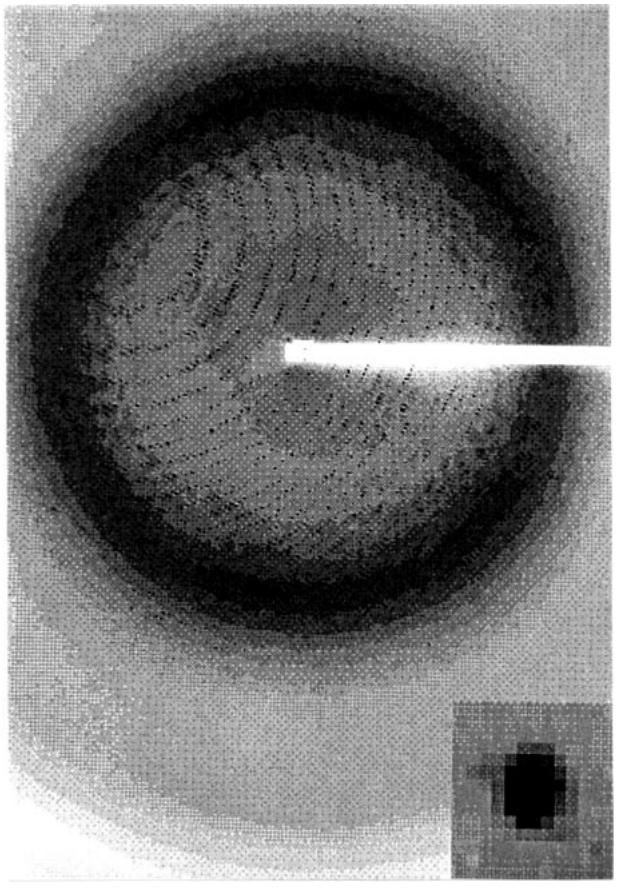

Fig. 2. Oscillation photograph of bovine heart cytochrome c oxidase. A magnified spot is shown in a frame. A half-value width of the spot is less than 150mm.

2.2. ACC deaminase

1-Aminocyclopropane-1-carboxylic acid (ACC) is a cyclic amino acid isolated from fruits. It is regarded as a key intermediate in the biosynthesis of ethylene, a plant hormone that affects diverse growing and developmental processes, including fruit ripening, leaf and flower senescence. ACC deaminase isolated from a yeast Hansenula Saturnus catalyzes the cleavage of ACC to a-ketobutyrate and ammonia by a cyclopropane ring opening reaction. The introduction of this enzyme into plants by gene technology as an inhibitor for ethylene biosynthesis has been proved to be useful in plant physiology; it provides a way to regulate ACC levels and ethylene biosynthesis. The PHMBS co-crystal of ACC deaminase belongs to a trigonal space group P3221(P3121), with a=b=80.1Å and c=247.2Å. An asymmetric unit contains one dimer of Mr= 69,000. The X-ray data was collected at the station BL41 XU using a single crystal at 100K (Fig. 3). The data was processed by HKL program package to give a final data set containing 37,871 unique reflections to 2.3Å (91% of expected reflections at this resolution) with an overall Rmerge factor of 4.5 %. Bijvoet difference Patterson map (using data from 15Å to 3.5Å) clearly shows two Hg sites (Fig. 4).

(Atsushi Nakagawa and Isao Tanaka)

2.3. FMN-binding protein

A protein isolated from Desulfovibrio vulgaris Miyazaki F (Mr = 13,700) contains a FMN group as a prosthetic group. The function or role of this FMN-binding protein is unknown. We believe, however, the high resolution X-ray analysis could elucidate mechanisms of this protein and/or the FMN group in atomic level. We have tried to collect higher diffraction data from a single crystal with dimensions of 0.2 × 0.3 × 0.2mm. The BL41XU beamline is very powerful for this purpose, which gives a limit of diffractions recorded on a large imaging-plate.

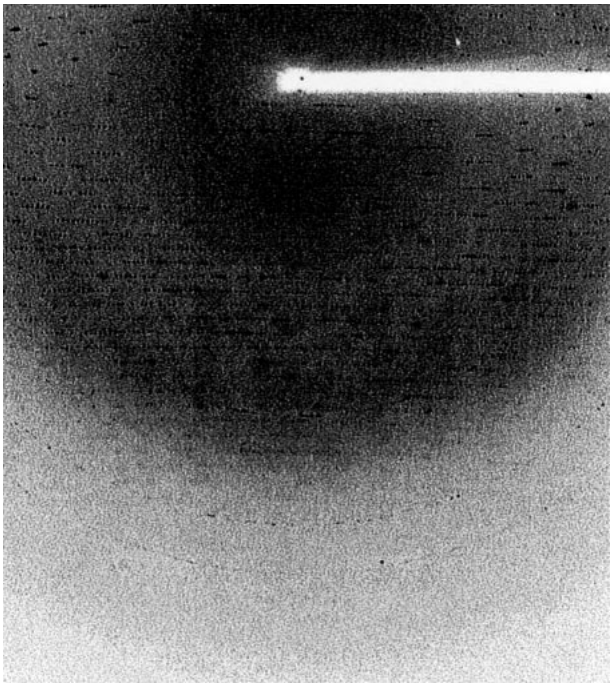

Fig. 3. Weissenberg photograph of 1-amino-cyclopropane-1-carboxylate (ACC) deaminase from Hansenula Saturnus co-crystallized with PHMBS. Oscillation range is 3.4 deg. (coupling constant is 0.6 mm/deg) and exposure time is 34 sec. Crystal is aligned so that the crystallographic c axis is in the spindle axis.

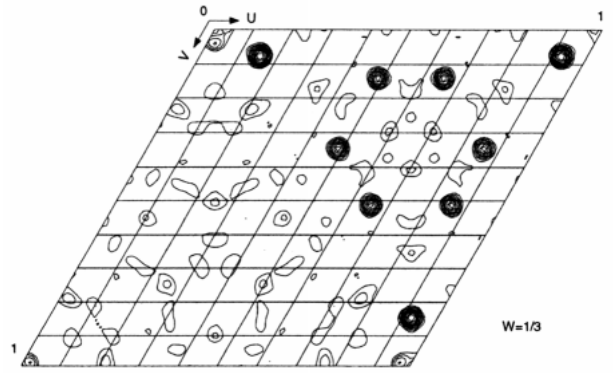

Fig. 4. Bijvoet difference Patterson map calculated from diffraction data collected at 0.9800Å. The interval of contour levels is 1.0 times the r.m.s. density of the map beginning at 1.0 times the r.m.s. Two independent peaks corresponding to two Hg sites are clearly shown.

Fig. 5 shows the diffraction pattern from the protein, and clearly presents spots on the edge of the IP (ca. 1.2Å resolution). With an oscillation angle of 15 deg and an exposure time of 15 sec, a total of 12 IP flames were obtained. Tertiary structure of the protein was determined at 2Å resolution by the MIR method, and the refinement of the model is now in progress.

(Yukio Morimoto and Noritake Yasuoka)

2.4. Photosynthetic reaction center

The primary process in photosynthesis involves absorption of light energy and its conversion into chemical energy. The reaction centers from purple photosynthetic bacteria are integral membrane proteins that accept energy from light-harvesting pigment protein complexes and start the conversion reaction from light to chemical energy. The reaction center from a thermophicpurple

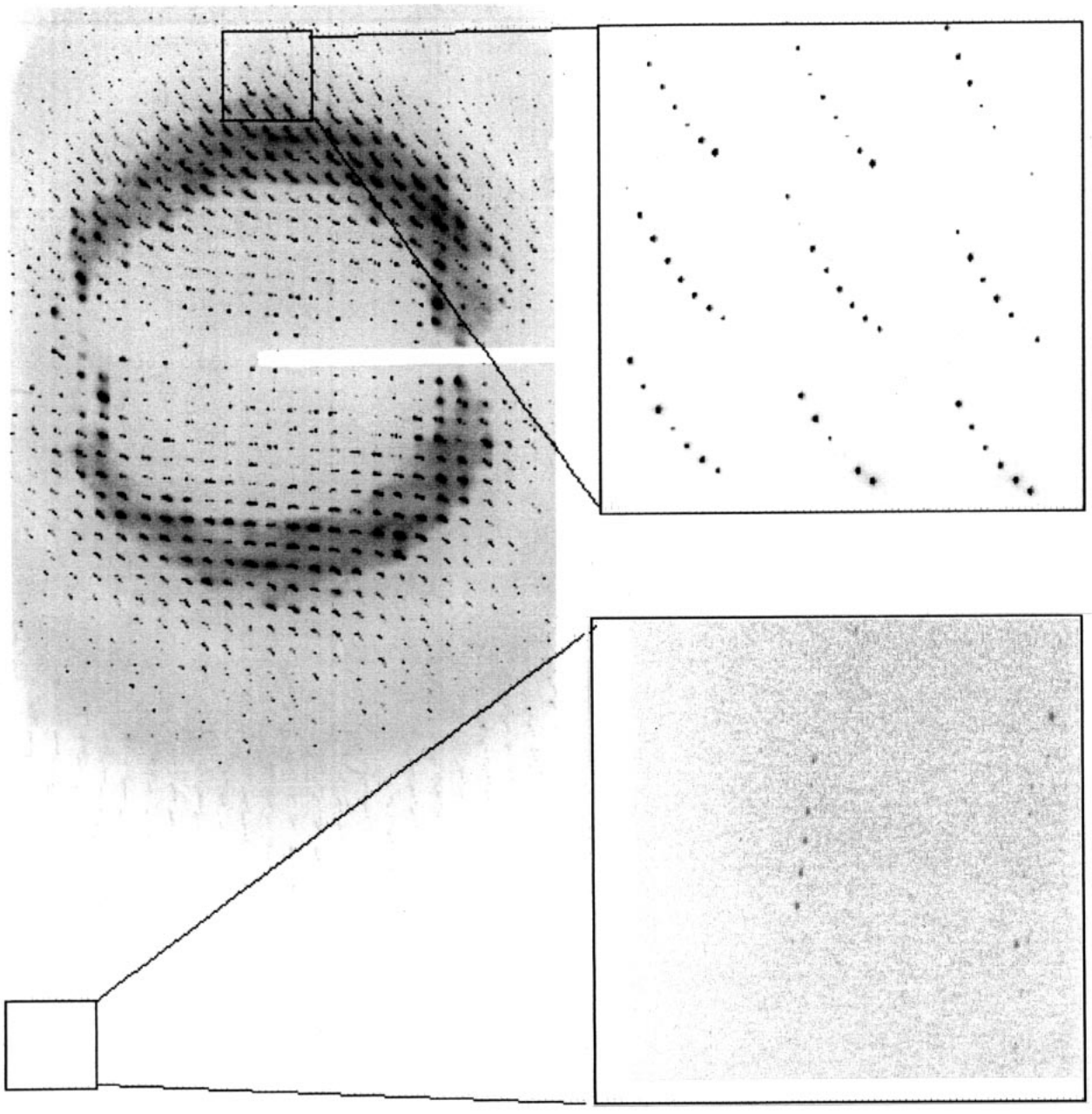

Fig.5. Diffraction pattern of FMN-binding protein obtained at BL41XU. Resolution on the edge of the IP is 1.2Å. Imaging-plate 40 × 80 cm, wavelength 1.0 Å camera length 560mm, IP translation distance 7.5 mm, exposure time 30sec.

bacterium, Chromatium tepidum (with an optimum growth temperature of 48-50˚C) shows a thermal stability up to 47˚C indetergent solutions. The crystals of this reaction center belong to the orthorhombicspace group $P2_12_12_1$, with a=134Å, b=201Å and c=85Å. An asymmetric unit contains one molecule of the reaction center complex with four subunits and several prosthetic groups (Mr=133,000). The X-ray diffraction pattern obtained from a crystal with the dimensions of 0.2 × 0.3 × 0.8mm at the station BL41XU is shown in Fig. 6. The diffraction pattern was recordedup to at least 1.8Å resolution. The crystal structures of two photosynthetic reaction centers from mesophilic purple bacteria were hitherto reported, in which the highest resolution of the structure was 2.3Å for the Rps.viridis reaction center. The present crystals of the C. tepidum reaction center will be able to give the most precise structure with the highest resolution not only for the reaction centers but also for the membrane proteins, in case of the use of the BL41XU beamline.

(Kunio Miki)

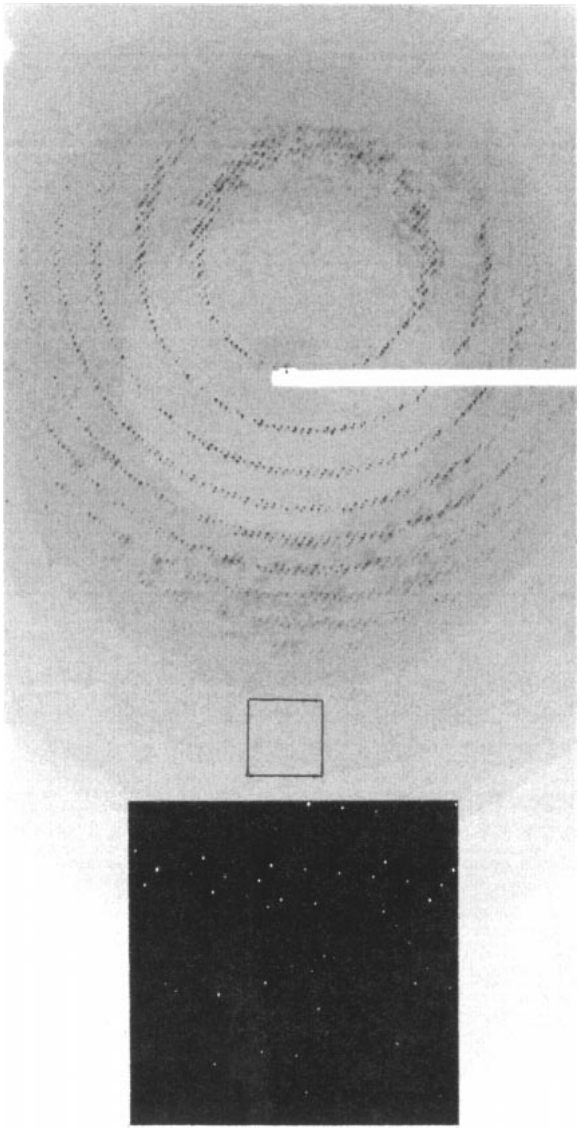

Fig.6. Oscillation photograph of the photosynthetic reaction center from C. tepidum.. Oscillation range is 1.0 deg. and exposure time is 10 sec. A magnified flame shows diffraction spots beyond 2Å resolution.

3. Concluding remarks

The Bio-Crystallography beamline (BL41XU) has been well-constructed and very excellent diffraction data from a variety of protein crystals have already been obtained. It was found from several preliminary diffraction experiments mentioned in the above section that the X-ray beam is very suitable for the MIR-OAS and high-resolution crystal structure analyses of the biological macromolecules including the membrane proteins. In the next stage, the automated on-line read-out system for the exposed IPs should be contracted to the IP diffractometer installed in the experimental station. This system will enable us to perform the complete MIR-OAS crystal structure determination, which is the authorized purpose of the Bio-Crystallography beamline. In parallel, we have to develop and improve the cryo-cooling conditions for each protein crystal, which is necessary in collecting the high quality diffraction data with this beamline. It is expected that a lot of valuable structures of the biological macromolecules will be elucidated by the use of this beam line.

(Kunio Miki)