Development of Cryo-cooling System for Cryogenic Protein Crystallography at the RIKEN Beam Line

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The beam line for protein crystallography in the RIKEN beam line at Spring-8 has been designed for the optimized utilization of the multi-wavelength anomalous diffraction (MAD) method to solve structures of proteins at atomic resolution [1]. In the MAD method, we have to measure small differences in Friedel pairs with high statistical accuracy. Hence, the beam line components, such as trichrometer [1], mirrors and goniometers, have to be controlled at high precision and, furthermore, degradation of protein crystals by X-radiation during experiments has to be minimized. It has been well known that the cryogenic experiments at the liquid nitrogen temperature is the most effective method to eliminate X-radiation damage of protein crystals, because generation of free radicals and temperature-rise, which cause the degradation of protein crystals, could be remarkably suppressed at low temperatures. By using cryogenic techniques we can collect reliable intensity data with high accuracy through fairly long time exposure of protein crystals to X-rays. In order to establish the technique, we have investigated and developed the following procedures;

1) introduction of anti-freezing reagents into crystals.
2) rapid freezing of crystals mounted on a special device.
3) preservation of crystals in liquid nitrogen.

Most protein crystals are broken by ice formation, becausethey usually contains water more than 30 % in its volume. Since the ice formation can be prevented by anti-freezing reagent such as glycerol, sucrose and polyethylene glycol, we introduce them into protein crystals by dialysis method with micro-cells. However, because these reagents are not necessaruly effective for all the protein crystals, we have to select a regent proper for a protein crystal before the cryogenic experiments.

In our system, a specially designed crystal mounting device was used for effective cooling of protein crystals by cryogens. Because the conventional capillary tube is notsufficient for effective heat transfer, we have developed a mounting device by slicing a capillary tube into thin rigs with a height of 0.1 mm [2]. And the other device, such as spatula mounting device [3] and free-standing thin films [4], are also available. These devices are further mounted on a head pins fixed on a small and strong magnet for quick alignment of protein crystals on goniometer during rapid freezing procedure.

For the rapid freezing, we have chosen low temperature nitrogen gas, liquid ethane and liquid propane as cryogens. Especially, the latter two were quite suitable for the rapid freezing, because there exists no bubbling in the interface between protein crystal and them. We have also developed a shutter mechanism for the utilization of cold nitrogen gas and rapid transfer mechanism of protein crystals from liquid ethane to the cold gas flow area after the completion of freezing. The temperature of frozen crystals are maintained with continuous flow of cold nitrogen gas by using the Cryostream Cooler (Oxford) during data collection. Development of the preservation method of protein crystals is now under way.

The performance test of the present system has been performed in our laboratory with a conventional X-ray generator and the R-axisIIc system (Rigaku) on some protein crystals. In the case of trypsin crystal frozen at 100 K, the resultant $R_{merge}I$ for reflections located between the Bragg spacing of 46.0 and 1.8Å was 0.049 and it was shown that our system was suitable for data collection for radiation sensitive protein crystals. We have succeeded to extend the resolution limit up to 2.5Å for a protein crystal, whose eresolution limit was 6Å at room temperature, by using our present system. We hope that the system will provide statistically good data in MAD experiments.

References

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