This Bijvoet difference, however, is still small and highly accurate data collection is essential. One of the experimental difficulties using longer wavelength is the increased absorption. Therefore we have developed a crystal mounting technique to eliminate absorption by the frozen cryo-buffer around protein crystal (Kitago et al., 2005), and the practical applicability of this mounting method was examined using several novel proteins at CrKα radiation of 2.29 Å (Watanabe, 2006). In order to utilize this mounting method at the synchrotron beamlines, we made this mounting tool compatible to the standard Hampton CrystalCap. With a special magnet base for this cap, it becomes possible to mount and remount frozen crystals as the standard CrystalCap. We have tested its applicability by sending several frozen crystals to the beamline BL13B1 at NSRRC, Taiwan. This mounting method is also very useful at synchrotron beamlines to mount tiny crystals that are difficult to center because of the lens-shaped frozen buffer in the cryoloop. In this development, we also use a loop made of a polyimide film microfabricated by photolithography.

[1] Kitago, Y., Watanabe, N. and Tanaka, I. (2005), *Acta Cryst*., **D61**(8), 1013-1021.
[2] Watanabe, N. (2006), *Acta Cryst*., **D62**(8), 891-896.

Keywords: SAD, longer wavelength SAD, crystal mounting method

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Application of a novel mounting tool using adhesive for protein crystals

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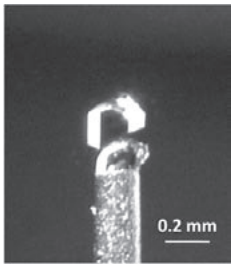
We have developed a novel mounting tool for protein crystals that directly captures a crystal from a drop with an adhesive (Kitatani *et al*., 2008). This tool has a loop-free structure and can reduce the amount of solution around the captured crystal in comparison with conventional loop-based mount tools. The extra solution around captured crystal not only degrades the quality of data-collection statistics but also disturbs the initial phase determination by the sulfur single-wavelength anomalous diffraction (SAD) method. The effect of reducing the solution in this tool is helpful in the sulfur SAD method. We have evaluated the effects of our tool on a quality and crystallographic data in the sulfur SAD method using CoK_α radiation.



Reference
[1] Kitatani T., Sugiyama S., Matsumura H., Adachi H., H. Yoshikawa Y., Maki S., Murakami S., Inoue T., Mori Y. and Takano K. (2008) *Appl. Phys. Express*, 1, 037002.

Keywords: biomaterials and biodevices, protein crystals, SAD

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Structural transitions and the evolution of protein crystal disorder during slow cooling

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We report measurements of the lattice perfection and unit cell volume of Lysozyme, Thaumatin, and Trypsin crystals as function of temperature during slow cooling (200 K/hour) from 300 K to 100 K. We demonstrate that these crystals can be slow cooled without formation of internal crystalline ice, even when no penetrating cryoprotectants are used. Most degradation of diffraction properties occurs between 240 and 180 K. In Thaumatin crystals, slow cooling produces low temperature diffraction properties that are as good as or better than those obtained with flash cooling or with cooling under pressure. In Lysozyme and Trypsin crystals, crystal order abruptly and dramatically degrades near 210 K. This dramatic degradation is associated with an apparent first-order phase transition signaled by an abrupt decrease in unit cell volume. We examine two possible origins for this transition: cold denaturation of the protein, and a transformation between low density and high density liquid water within the nanometer-sized channels of the crystal.

Keywords: cryocooled crystallography, structural phase transitions, biological macromolecular crystallography

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The temperature dependence of radiation damage to macromolecular crystals

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We have studied how the rate of global radiation damage to Lysozyme, Thaumatin, and Trypsin crystals varies with temperature. The radiation sensitivity of these proteins decreases by a factor of 30 to 50 between T=300 K and 100 K. Most of the decrease occurs between 220 K and 180 K, and we attribute this to a glass transition in the protein + solvent system. Addition of 40% glycerol raises the temperature for the drop in radiation sensitivity by ~10 K, consistent with the measured shift in the glass transition.

Keywords: radiation damage, biological macromolecular crystallography, glass transition

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Manipulating protein microcrystal with optical tweezers based on lensed fiber probes

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In many synchrotron facilities, X-ray microbeam will be utilized for protein crystallography. It will be possible to collect diffraction data from a protein microcrystal with the size in the range from 1

micro meter to 10 micro meter. Until now a protein crystal is picked up manually from a crystallization droplet using a cryoloop and is mounted on a goniometer head. However it seems to be impossible to manipulate the protein microcrystal by hands, because the protein microcrystals are very small and fragile against a shock. So we are developing an automatic microcrystal pick-up system. In the system, we applied optical tweezers to manipulate the fragile protein crystal. It was reported that the optical tweezers at the near-infrared region traps and manipulates a cell without critical photodamage. We tried two kinds of optics for optical tweezers. One had a condensing lens, the other had a lensed fiber probe, which focused the laser on the object. The optical tweezers with the condensing lens succeeded in trapping of protein crystals with the size in 100 micro meter range. X-ray measurement of the trapped crystals indicated that laser trap with 1064 nm wavelength hardly affected the result of X-ray structural analysis. On the other hand, the optical tweezers with the two lensed fiber probe could manipulate the protein microcrystal with lower emission power. The lensed fiber probe was smaller than former, which had an advantage to be operated in the crystallization droplets at various crystallization plates.

Keywords: protein microcrystal, optical tweezers, crystal manipulation

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A new understanding of radiation damage at cryogenic temperatures

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Radiation damage is one of the remaining bottlenecks in structural biology. In the literature many aspects of radiation damage are very well described, however the underlying mechanism responsible for the loss of diffracting power of the crystals remained unknown so far. To get a deeper insight into the processes involved we have investigated radiation damage by combining traditional X-ray data collection with small angle X-ray scattering over a broader temperature range from 5 K to 160 K. All experiments were carried out at the protein crystallography beamline X06SA at the Swiss light source (SLS) equipped with a Pilatus 6M pixel detector. For all crystals individual increase or decay rates for different quality parameters with dose were determined and analyzed as a function of temperature. Interestingly a unexpected temperature dependence for all these parameters was observed. We could identify an optimal data collection temperature, where radiation damage is reduced by about 30% compared to data collection at 100 K, were most experiments are performed today. To gain information about the length scale of the disorder phenomena we performed small angle X-ray scattering experiments on cubic insulin crystals at the same experimental conditions and temperatures as used for the data collections. Analysis of the diffuse scattering signal lead us to two different mechanisms responsible for the loss of diffracting power. We could identify hydrogen gas, formed during irradiation of biological samples with X-rays, as the main damaging agent at cryogenic temperatures. Our findings are in good agreement with observations from cryo-electron microscopy and explain the basic mechanism of radiation damage at cryogenic temperatures for the first time in a conclusive manner.

Keywords: radiation damage, cryocooled crystallography, small-angle scattering

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Crystal structure of the peptidoglycan recognition protein at 1.8 Å resolution

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The mammalian peptidoglycan recognition protein-S (PGRP-S) binds to peptidoglycans (PGNs) which are essential components of the cell wall of bacteria. The crystals of PGRP-S belong to orthorhombic space group *I*222 with a = 87.0Å, b = 01.7Åand c = 162.3Å having four crystallographically independent molecules in the asymmetric unit. The structure has been determined with molecular replacement method and refined to an R_{cryst} and R_{free} factors of 0.225 and 0.247 respectively. Overall, the structures of all the four molecules are identical. The folding of PGRP-S consists of a central β-sheet with five β-strands, four parallel and one antiparallel and three α-helices. This protein fold provides two functional sites. The first of these is the PGN-binding site, located on the groove that opens on the surface in the direction opposite to the location of the N-terminus. The second site is implicated to be involved in the binding of non-PGN molecules, it also include putative N-terminal segment residues, (1-14) and helix α2 in the extended binding. The structure reveals a novel arrangement of PGRP-S molecules in which two pairs of molecules associate to form two independent dimers. The first dimer is formed by two molecules with N-terminal segment at the interface in which non-PGN binding site is completely buried whereas the PGN- binding sites of two participating molecules are fully exposed at the opposite ends of the dimer. In the second dimer formed by another set of two molecules in which the PGN binding sites are buried at the interface while the non-PGN binding sites are located at the opposite surfaces of the dimer. This form of dimeric arrangement is unique and seems to be aimed at enhancing the capability of the protein against invading bacteria.

Keywords: innate immune system, pgrp, pattern recognition

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Structural insights into an affinity-based selection of virus-specific public T cell receptors

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To be protected against intracellular pathogens, vertebrates have developed an adaptive immune response based on a large number of T lymphocytes. Each T cell clone displays an Ig-like receptor (TCR) specific for antigens bound to Major Histocompatibility Complex (MHC) molecules, present at the Antigen Presenting Cell surface. Although the thymic selection generates a diverse naive repertoire