

strategies to collect dataset efficiently for the S-SAD method, (i) lowering the measurement error using high brilliant short wavelength X-ray, (ii) augmenting the anomalous signal using long wavelength X-ray. To validate these strategies, we experimented on the S-SAD method with standard samples at two synchrotron beamlines, SPring-8/BL41XU and SAGA-LS/BL15. (i) We have collected datasets from standard samples (insulin, thaumatin and lysozyme) using various wavelength between 0.71 to 1.90 Å at the SPring-8 undulator beamline BL41XU. We succeeded in the determination of the phases and building the molecular models of insulin from dataset collected in 0.9 – 1.9 Å wavelength, thaumatin from 1.5 - 1.9 Å and lysozyme from 1.5 - 1.7 Å, respectively. In addition, it ascertained that the dataset collected in longer wavelength allowed the phase determination and model building from lesser redundancy. (ii) SAGA-LS/BL15 has a bending magnet as a light source, and it is able to use X-ray between 0.54 to 5.90 Å wavelengths. The preliminary X-ray diffraction experiment of insulin crystals using 1.5, 2.3, 2.6 and 2.9 Å wavelengths was performed at this beamline. The R_{merge} of the collected datasets were about 4 - 7 % in all wavelengths, and the molecular models could be built automatically from dataset in 1.5 and 2.3 Å wavelengths by S-SAD analysis. From the datasets collected using 2.6 and 2.9 Å wavelengths, we could determine the position of sulfurs and the phase but not build their models automatically due to the less of the resolution.

Keywords: protein crystallography with synchrotron radiation, anomalous dispersion methods, sulfur

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Sulphur SAD (S-SAD) phasing using CoK_α radiation

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The S-SAD phasing is extremely useful for structure determination of a novel protein especially when its heavy atom derivatives are difficult to prepare. For S-SAD phasing, CrK_α radiation has generally been used in a laboratory because its wavelength (2.2909 Å) is closer to the absorption edge of sulphur (~5 Å) than that of CuK_α radiation. However, precise measurements of anomalous signals using X-rays with such a long wavelength is sometimes difficult due to errors originating from numerous sources such as air dispersion, absorptions by solvent around a crystal, a mounting loop, and components of an instrument such as a window material. Furthermore, with a plate detector, it is cumbersome to collect high resolution data that are necessary to reach the final structure. One can minimize the camera length to record high angle reflections, however those reflections will suffer severe errors by absorption and obliqueness. The optimal wavelength for S-SAD phasing may lay somewhere in-between CuK_α and CrK_α . Therefore we have developed an X-ray generator producing strong CoK_α radiation (1.7902 Å) based on an FR-E Superbright (Rigaku) and built a S-SAD system around it. The final system consists of an FR-E Superbright DW (Cu/Co) (Rigaku), a VariMax Co (Rigaku Americas) and an R-RAXIS VII/HTC (Rigaku). To reduce the absorption, we eliminated the black shielding film of the image plate detector. In order to evaluate the Co system, we tried structure analysis of four commercially available proteins: Insulin, HEW-Lysozyme, Glucose isomerase, Trypsin and Thaumatin. We will show these experimental data at the conference.

Keywords: SAD, protein phasing, single anomalous diffraction

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Development of computer software for general area detector diffraction system(GADDS)

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We have developed the software which determines the orientation of the single crystal using General Area Detector Diffraction System(GADDS). The developed software is possible to determine the orientation for all kinds of crystal system and to analyze the single crystal when Detector's position was laid down at every directions against the beam direction. In case a uncertainty of the position of diffraction spots is below a millimeter, the orientation can be determined the right way by the least squares fitting. The software can previously forecast a diffraction situation by inputting the data which are a crystal system, a lattice constant and suchlike. The software offers a method that turns the single crystal determined the orientation toward the direction wanted by user. GADDS Analysis Program developed with C++ programming language can be utilized for the instruments of X-ray diffraction as well as Analysis of Laue-Film.

Keywords: Laue film, diffraction pattern, analysis software

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Structural changes of reaction centre from *Bl. viridis* revealed by time-resolved Laue diffraction

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Reaction centre of *Bl. viridis* (RCvir) was the first membrane protein from which high resolution diffraction data was ever obtained. The structure showed three subunits and the photosynthetic pigments which were ordered in a two fold symmetric arrangement. Further, an electron pathway across the membrane was proposed, initiated by capturing a photon by the special pair, a bacteriochlorophyll dimer located on the periplasmic side. This absorption generates an electron, which migrates through the membrane to the movable ubiquinone, the final electron acceptor. After reduction to ubiquinol, it will then be released into the membrane. Even though the basic structure of RCvir is known, detailed structural information about how light gets converted into chemical energy is still missing. This lacking information is of major interest, since RCvir has high homology to photosystem II of higher plants. If one gets more insight into the photocycle of RCvir one could use this information and apply it directly to eukaryotic systems. One way of exploring the reaction path is to perform a time-resolved laue experiment where the photocycle is initiated with a laser and electron transport is monitored with a polychromatic x-ray beam. The illumination is very demanding for the crystal and thus robust crystals are an absolute requirement for the experiment. So, we crystallized RCvir using the sponge phase crystallization method and a type I crystal form

was obtained. The crystals endured the high demands of the time-resolved laue experiment and several datasets, ground state and illuminated, diffracting to high resolution were collected. From this data difference maps were calculated which revealed interesting details including a tyrosin residue corresponding to tyrZ of plant photosystem II.

Keywords: time-resolved laue diffraction, membrane protein, lipidic-sponge phase

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XAFS and XRF studies of anti-bacterial ceramics using synchrotron radiation

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Recently anti bacterial techniques have been spread into daily commodities with improving consciousness of health. There are several kinds of anti bacterial consumers, silver courting or silver ions are most common anti bacterial chemicals in Japan, however, the resource of silver is limited and it became so expensive to be used for daily commercial item. In addition, the anti bacterial effects on silver have been unclear because of difficulties of in-situ chemical state analyses for these systems in ordinary analytical methods. In order to elucidate the anti bacterial mechanism of silver and develop new anti-bacterial material instead of limited silver, XAFS and XRF analyses using Synchrotron Radiation were applied for the sanitary wares and anti-bacterial tiles which are the products of INAX corporation, Aichi, Japan. For the comparison, other anti-bacterial tiles containing Zn were also examined by XAFS analyses. Each specimen was cut into 1cm x 1cm blocks to be used for analyses. Crystalline materials in the sample were examined by XRD, however, no crystalline staff was detected. From elemental analyses using EPMA, a content of silver in sanitary ware was about 0.08wt% and that of zinc was about 15wt%. Then XAFS measurements of the silver in anti-bacterial stuffs were performed for K-edge at BL-01B1 in SPring-8, and for L-edge at BL9A in Photon Factory, KEK. XAFS measurements for zinc were performed at BL12C and BL9A in PF. The result of XAFS measurements, chemical state of the silver in the sanitary ware was mono-valent, and silver was coordinated with about two oxygen in 2.22Å. On the other hands, zinc in the anti-bacterial tiles was divalent and there local structure was similar to Zn₂SiO₄ which is coordinated with four oxygen in 1.96Å

Keywords: anti-bacterial materials, synchrotron radiation, XAFS

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Local atomic structure of iron in Fe-chitosan complexes, determined by XAFS

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Chitosan is natural polymer, the N-deacetylated product of chitin which is obtained from crustacean shells. It possesses attractive

for nowadays technology physical and mechanical properties: easy biodegradation, biocompatibility and bioactivity. The most important aspect is its ability to chelate metal ions, including heavy and toxic ones. In presented paper the local atomic structure of iron in the Fe-crosslinked chitosan and N-carboxylmethyl chitosan were investigated in the light of their potential biomedical applications. From the performed advanced x-ray absorption fine structure (XAFS) analysis the coordination till the third sphere was determinate. Fe atoms in the investigated samples were penta-coordinated and appeared in Fe⁺³ ionic state. In Ch-Fe-CL in the first sphere two kinds of Fe coordination was found. Several Fe atoms are localized in the second coordination sphere. The third sphere was composed from six oxygen atoms. Knowing that amine group should come from the single chitosan monomer at least two or three monomers (or even polymers) should be bonded to each Fe atom and each of this polymer has in turn built-in Fe atom. In N-CM-Ch-Fe only one Fe neighborhood was found, three oxygen and two nitrogen atoms. Other Fe atoms were identified neither in first nor in second coordination sphere, but third coordination sphere indicates the presence of Fe atoms. In the second sphere three carbon and two oxygen atoms were identified. This provides the evidence that carboxyl group is an active one. These local atomic order were confirmed qualitatively by Mössbauer spectroscopy. This work was partially supported by national grant of Ministry of Science and High Education N202-052-32/1189 and by DESY and the EC under Contract RII3-CT-2004-506008 (IA-SFS)

Keywords: XAFS, chitosan, coordination

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100-picosecond time-resolved X-ray absorption fine structure of Fe^{II}(1,10-phenanthroline)₃

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Studying photo-induced molecular dynamics in liquid with subnanosecond time-resolution gives a information for understanding fundamental chemistry, biology and also for developing new materials and devices. Monitoring the dynamic phenomenon requires a sensitive tools to investigate the electronic state and the structure with atomic resolution[1],[2]. Previously, we have reported the success in measuring the photodissociation of ligands in NiTPP system in solution[3]. Here, we have performed time-resolved X-ray absorption fine structure on the spin-crossover complex Fe^{II} tris-(1,10-phenanthroline) dissolved in aqueous. In this system, excitation by femtosecond laser pulse of 400nm induces the spin state transition from low spin to high spin one as a result of the photo-induced expansion in the Fe-N bond length similar to other spin crossover systems[4]. All measurements were performed in fluorescence method at the iron K-edge on the undulator beamline NW14A at the Photon Factory Advanced Ring[3]. Obtained results clearly demonstrate the success in probing both structural and spin state changes induced by 400nm femtosecond laser pulse excitation with