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The native (Sulfur) SAD method in photon factory

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Crystallography has been a major method to determine 3D structures of biological macromolecules at atomic resolution. While a new method with cryo-EM is becoming another major technique for the 3D structure analysis, crystallography still has some advantages. Recently, many crystal structures of biological macromolecules are determined by MAD/SAD method with seleno-methionine proteins (SeMet-proteins). Since selenium has an X-ray absorption edge near 1Å, it is convenient to utilize in the MAD/SAD method. While this technique is useful, crystallographers need to prepare SeMet-proteins. If we can develop a method to solve the phase problem without using SeMet-proteins, it would be highly useful for crystallographers. So, we have tried to develop the native SAD (or sulfur SAD) method, in which anomalous signals from sulfur atoms in the native protein are utilized. However, there are some problems in the native SAD method. First, sulfur gives only weak anomalous signals with X-ray typically utilized in protein crystallography (X-ray wavelength of around 1Å). To increase the anomalous signals, we need to use a longer wavelength X-ray than usual. However, since a longer wavelength X-ray shows significant absorption by air, solvent, protein etc., data quality is degraded by the absorption. The native SAD method, therefore, requires a specific system for high quality data collection. To achieve routine utilization of the native SAD method, we have developed a beamline (BL-1A) dedicated for the native SAD method. In BL-1A, we can utilize a long wavelength X-ray (1.9-3.5 Å). Furthermore, the goniometer and X-ray detector are installed inside a He chamber to prevent the absorption problem. Our system enables us to solve crystal structures of proteins by the native SAD method. In the presentation, we will present several examples of crystal structure determination with native SAD. Also, we will mention our unique method for crystal freezing to collect high quality diffraction data required in native SAD experiments.



Figure1: BL-1A of Photon Factory dedicated to the native SAD phasing

Biography

Toshiya Senda has completed his PhD from Nagaoka University of Technology (Niigata, Japan) in 1995. He was a Research Associate in Nagaoka University of Technology (1995-2001) and a Senior Researcher in Institute of Advanced Industrial Science and Technology (2001-2012). Now, he is the Director/Professor of Structural Biology Research Center of High Energy Accelerator Research Organization (KEKI) in Japan. He was awarded the CrSJ (Crystallographic society of Japan) award in 2014 (Structural biology studies of CagA from *Helicobacter pylori* and histone chaperon CIA/ASF1).

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