A comprehensive strategy for quick determination of protein structures by MR-native SAD method

Miki Senda[1], Atsushi Kawaguchi[2], Toshiya Senda[3]

[1]High Energy Accelerator Research Organization (KEK) [2]Department of Infection Biology, Faculty of Medicine, University of Tsukuba, [3]Structural Bio Research Ctr Inst of Materials Structure , High Energy Accelerator Research Org,

miki.senda@kek.jp

A special advantage of protein crystallography is the quick structure determination of target proteins. The high-throughput structure determination of protein-compound complexes is critical for pharmaceutical and life sciences. To achieve rapid structure determination, we need to overcome two bottlenecks in protein crystallography, crystallization and phasing. We have developed several methods for obtaining good crystals and reported them in ACA meetings (1, 2, 3). Here, we present a useful method for phasing: the MR-native SAD method using a diffraction data collection system with low-energy X-ray, which has been developed at PF in KEK. It is relatively easy to obtain initial phases by the molecular replacement (MR) method using a starting model from PDB or AlphaFold2. However, since the starting model is not perfect, model bias arising from the imperfect model frequently hampers quick model building and crystallographic refinement. So, we have combined the MR method and an experimental phasing method using anomalous diffraction from sulfur atoms (native SAD). While a typical MR-SAD method needs derivative (typically Se-Met proteins) crystals for measuring anomalous signals, the MR-native SAD method does not need derivative crystals. Since we have developed a beamline for the native SAD method, it is possible to use anomalous diffractions from sulfur atoms for phasing. Diffraction data collection using low-energy X-ray of 1.9 Å or 2.7 Å wavelength is possible with BL-1A, which provides high-quality data for the SAD phasing (4). A crystal shaping machine can also be utilized to improve diffraction data quality (5). However, since the determination of the substructure is frequently difficult in the native SAD phasing, initial phases from the MR method are valuable for the substructure determination. Here, we present several examples of crystal structure determination using the MR-native SAD method (6, 7). MR-native SAD frequently gave a high-quality model without manual model building, even when the MR method gave a poor model that was too difficult to fix problems manually (Figure 1).

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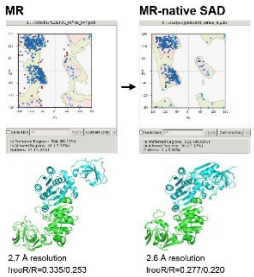

Figure 1