Crystallographic analysis of proteins related to drug design VI

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The therapeutic agents for human diseases exert the pharmacological efficacy via modulating certain target's function(s), and the target is often protein(s) as the major functional machinery in living organisms including human. The agents, which possess to modulate such target protein's function *in vitro,* are possible candidates for therapeutics *in vivo*. Thus the atomic resolution structures of the proteins, which have potential linkage to a human disease, are the indispensable information to design the compounds for the therapeutic candidates.

We have been trying to solve the structure of viral proteins for development of anti-viral agents. Viral protein for the objective was overexpressed in *Escherichia coli*, and was purified to homogeneity. The protein was crystallized according to usual procedure, but the crystals of the protein were not grown routinely and easily twinned. We collected the duplicated diffraction data sets using several X-ray sources including SPring-8 BL24XUA and BL45XU, and KEK-PF BL6B as well as our *in house* system. Since one crystal lattice is longer than 250Å, we corrected the diffraction data set up to 3.0 Å at the detector distance 500mm at BL24XU-A. The data sets were usable for crystallographic calculation only temporary because of the limited high resolution with the lack of needed low-resolution reflections due to large shadow of big beam stop when collected. The beam of BL24XUA was fine but not so intense using the 0.1mmϕ collimator, and we used the longer exposure time even more than the RAXIS IV read-out duration (4 min.) in our data collections.

In our opinion, BL24XU-A beam-line is not excellent for protein crystallography in our criteria but still usable only for molecular replacement methods using high quality crystals with normal crystal size (more then 0.1mm in the least dimension) and cell lattice (up to about 100 Å). Because: (1) The wavelength (0.83Å) was too short for common heavy atom derivative to collect anomalous data (around 0.95Å is preferable). (2) Beam-stop was too large to collect lower reflections than 8 Å (as low as 15Å reflections should be collected at least). (3) The computer system for data collection was too unusually unstable to collect without supervision after starting auto-data collection. (4) The limitation for higher resolution data set was presumed due to the low intensity of X-ray and the long turn-around time for SR data collection system.

We hope these points have been solved already or will be clear soon or later.

In our objective, we succeeded to solve the structure of the protein using the data sets which were collected other beam sources.