The flexibility of protein molecule in terms of the crystallography of DHFR mutants

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The flexibility of protein molecule plays an important role in its function. We are now studying the structure-flexibility-function relationships of *Escherichia coli* dihydrofolate reductase (DHFR). The flexibility of the whole molecule can be characterized by measuring adiabatic compressibility. From this criterion, DHFR is classified as "soft" protein. We have already analyzed the three dimensional structures of its mutants at 67 site (G67V, G67L and G67A), using the photon factory diffraction data. We found obvious difference in the distributions of the internal cavities among these mutant structures. Interestingly, the mutant having a large amount of cavity showed a large compressibility, demonstrating an important role of cavities in the protein flexibility.

This time, we targeted on to another position site 121, which is far from the active site residue by 19 Å. We attempted to crystallize three mutants, G121V, G121A and G121S but these crystals did not grow up so large as those of G67 mutants. Therefore, in order to utilize the high performance X-ray analyses of super synchrotron radiation beam, the diffraction data were collected at BL41XU beamline using the small crystals of 0.08mm width and 0.03mm thickness. The data for G121V and G121S crystals were collected at room temperature in the glass capillary tube, and the data for G121A at 100K using a cryocrystallography equipment. The G121V crystal belongs to the space group $P6_1$ with cell parameters a=93.0 Å and c=74.4 Å, which contains two molecules per asymmetric unit. The diffraction image was processed using AUTO software, and partially using MOSFILM software, up to 2.2 Å resolution.

For the G121V mutant data, molecular replacement was applied with AMoRe followed by the crystallographic refinement using the program X-PLOR. The R-factor is now converging to 18.9 % (R_{Free}=28.8%). The changes in electron densities were clearly recognized by replacing Gly to Val at site 121. The loop involving site 121 is considered to be strongly related to the reaction mechanism because this loop is adjacent to the M20 loop (residues 14-22) which locates beside the active site Asp27. The data processing and analysis of G121S and G121A are now in progress. We are planning to elucidate the role of loops in the protein dynamics by comparing the cavities and compressibility as done for the mutants at site 67.