X-ray Structural Analyses for a Series of Mutant Human Lysozymes

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We have studied stabilization mechanism of protein structures using a series of mutant human lysozymes. We plan to examine more than 1000 mutant proteins. The stabilities of the mutant proteins are investigated by differential scanning calorimetry, and their structures are determined by X-ray crystal analysis. From these data, contribution of stabilizing factors, such as hydrophobic effect and hydrogen bond, to the conformational stability of a protein could be evaluated. It would provide with not only the interpretation of protein stabilization mechanism but also the elucidation of the principle of how the tertiary structures of proteins are coded for in their amino acid sequences. Up to date, more than 50 mutant protein structures, of which crystals were large (> 0.3 x 0.3 x 0.2 mm^3), have been already analyzed. However, for some mutant proteins the structures have not been determined because their crystals are too small.

In order to understand the protein stabilization mechanism, we also have an interest in the problem of protein hydration. So, the data collection at low temperature (100 K) are needed for the mutant proteins substituted at residues on the protein surface.

At BL41XU of SPring-8, we could collect the data at 100 K for two mutant human lysozymes, S80A and V74A. The crystal of S80A, one of the mutant proteins constructed in order to investigate the role of hydrogen bond in protein stability, was very small (0.05 x 0.05 x 0.1 mm^3). The data set of S80A consisted of two oscillation and 18 weissenberg photographs. However, it had large mosaicity due to the low quality crystal or the failure in cooling the crystal. The crystal size of V74A human lysozyme substituted at Val 74 by Ala on the protein surface was 0.1 x 0.1 x 0.2 mm^3. The data set of V74A included two oscillation and 19 weissenberg photographs. It was the good data set. The refinement of the V74A crystal structure is in progress. We have already collected the data of V74S mutant protein at BL41XU of SPring-8 at 100 K in 3rd cycle of March, 1998. These structures would reveal the motion of solvent molecules on protein surface.