X-ray Crystallographic Analysis and Protein Engineering

Lab. Applied Structural Biology

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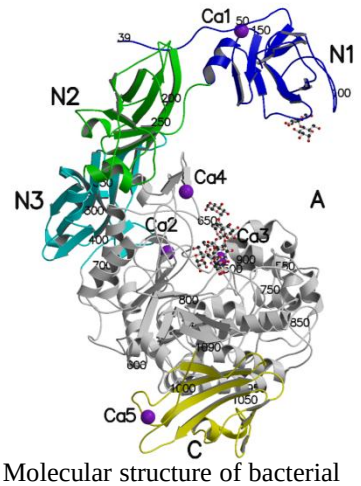
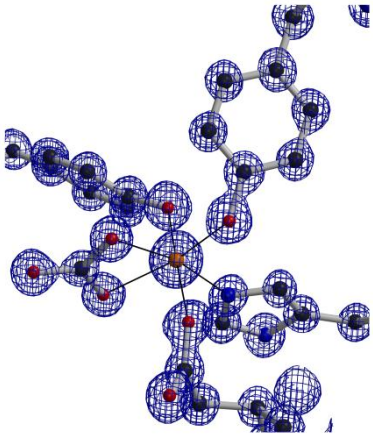
Information on genes has been accumulated through the analysis of the genomes, but the practical "function" of individual proteins in the living organisms is not yet thoroughly understood. Polypeptides, polymers of amino acids which are biosynthesized from gene information, have unique "structure" and after that, perform their "function" as proteins such as enzymes, muscle and hair. In order to understand the actual life "function" of proteins in the living organism, it is important to elucidate the relationship between the "structure" and "function" of proteins by protein structure analysis.

Structure Determination of Proteins and Enzymes

Using X-ray crystallographic analysis, we have determined 3D "structures" of many proteins (Egg white proteins, plant seed proteins, lectins and so on) and many enzymes (amylases, pullulanase, poly-saccharide lyase, and so on). Furthermore, proteins forming good crystals such as ovotransferrin could be applied for sub-atomic resolution X-ray crystallography and neutron crystallography in order to determine the positions of hydrogen atoms.

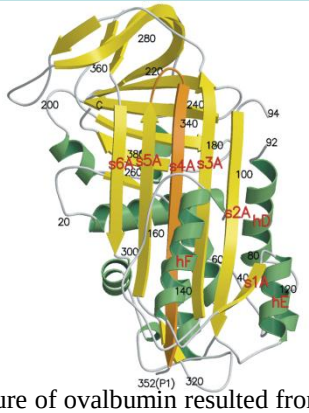


Molecular structure of bacterial pullulanase consisting of 5 domains.



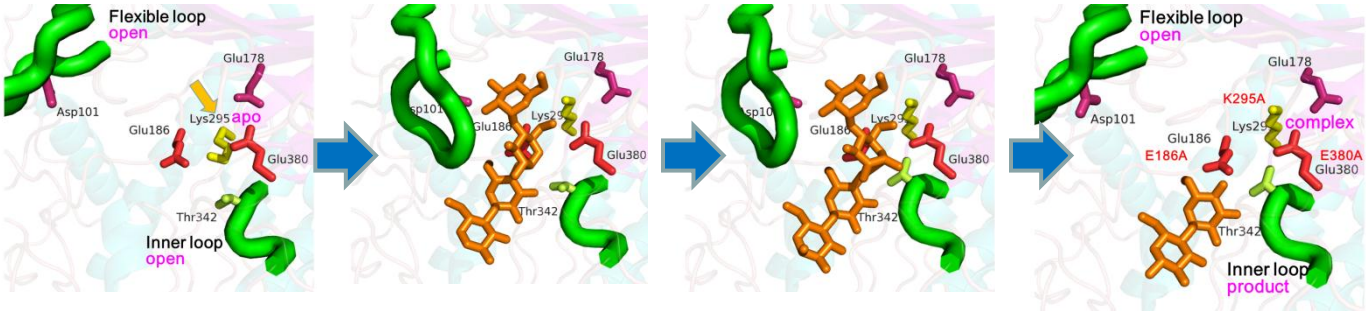
Electron density map around iron in ovotransferrin at 0.86 Å resolution.

Functional Analysis and Protein Engineering based Structure Analysis



Structure of ovalbumin resulted from loop Insertion which occurred by genetic engineering

Proteins actually exert their "function" by transforming their original "structure". By tracing this "structural" changes by X-ray crystallographic analysis, molecular mechanisms for the "function" of proteins could be understood. Hereby protein engineering toward improvement of original "function" should be possible. Currently, we are working on several projects such as, modification of β-amylase in optimal pH, conversion of pullulanase in its substrate specificity, furnishing a serine protease inhibitor activity to egg white ovalbumin, and dissection of enzymatic activity on lysozyme.



The conformational changes of two loops and Lys295 in the active site of β-amylase.

Keywords

Structural biology, X-Ray Crystallographic analysis, Protein engineering, Protein crystal growth, Microgravity

Recent Publication

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