examined the structure of AChR in the desensitize state by cryoelectron microscopy at 2.3 nm resolutions. This study gave new understanding to us. The three dimensional structure indicated that one of five subunit considering as delta subunit might become deformed to cover the inside pore of AchR, block the gate and so the AchR should be desensitized.

2P-026 高純度に精製された三成分のセルラーゼのX線結晶構造解析による詳細な構造比較

Comparison of the atomic structures of cellulase crystallized from three components separated by high-resolution chromatography

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It is well known that purify of the sample is one of the most important parameter for success of crystallization. Moreover, less impurity often gives better quality crystals. In this study, we used cellulase extracted and purified from *Aspergillus niger* a member of 1,4- β -D-glucan cellobiohydrolase as a model protein for high resolution X-ray crystallography. At the final step of the purification, we found that cellulase sample could be separated to three isomers by using Mono-Q HR5/5 column (GE Healthcare) with the slow gradient of NaCl for elution. These isomers indicated the same pattern in a SDS-PAGE analysis, however the each sample indicate different band in the Native-PAGE analysis. Each components of the cellulase give three different shaped crystals. Two data sets were collected for high- and low-resolution data to avoid the saturation of high intensity diffraction and overlaps of diffractions from each crystal. The diffraction data were observed up to 0.98 Å, 0.95 Å and 0.91 Å resolution from each crystal of the different components. From our previous studies, crystals obtained from mixed sample diffracted only up to 1.1 Å resolution. These results indicate that the sample quality strongly affect to the quality of crystals. Refinements of the atomic structures of cellulase from each crystals of the different components is now in progress and the results will clarify the relationship between quality of the sample and quality of the crystal from the atomic structures.

2P-027 精密化プロセスを含めたタンパク質-タンパク質ドッキング手法の開発

Protein-protein docking system with refinement stage

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Protein-protein docking prediction inherently have to deal with the huge degree-of-freedom (DOF) originated mainly from the freedom of motion in large molecules. The strategy often used to handle the vast search space caused by the huge DOF is to use multiple stages, i.e., an initial stage with rough calculation, followed by a refinement stage with finer calculation. By following this strategy, we have developed a multi-staged protein-protein docking system composed of a fast initial stage docking for rigid body model and a refinement stage with rescoring, reranking, clustering, and structural refinement.

The initial stage docking searches the six dimensional space of rigid body model by using the technique we have already developed in the past, namely, using series expansion in the spherical polar coordinates for fast computation of rough scoring functions. The refinement stage then processes top-ranked candidates generated by the initial stage with the following three processes: (1) The rescoring process calculates the finer scores based on van der Waals energy, electrostatic energy, and desolvation energy, and then reranks the candidates. (2) The clustering process then brings close ones in the top-ranked candidates together to reduce the number of candidates. (3) The structural refinement process refines the structures of the candidates by minimizing the physical energy. We will report the result of the multi-staged system and the detail of the effect of the refinement stage in the presentation.

2P-028 カルパインドメインVIの立体構造上のイントロン位置 Intron-Positions in the Tertiary Structure of Calpain Domain VI

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The significance of intron for the protein structures is considered and an interesting observation is reported here as to calpain domain VI.

Introns have been thought have no-meaning in the protein structure since they are splice out from mRNA before the translation process. However, introns in DNAs are the joints for both the alternative splicing and the exon shuffling, which could produce a new and bigger gene from smaller genes during evolution. Some introns encode proteins necessary splicing or transacting control of gene expression. In addition, the for self structures of some introns are found to have physiological meaning for alternative splicing and the rapid degradation of incorrect mRNAs. Calpain is a cysteine protease that needs Ca ion for the activation.

This hetero dimer enzyme contains thiol-protease site as the catalytic site and calmodulin because and cuts the peptide bonds not for digestion but for the activation of various proteins. The functions of the substrate proteins are known to be the key proteins for cellular processes, signal transduction, apoptosis, regulation of cell cycle, reconstruction of cytoskeleton, muscle homeostasis, nerve growth and so on. The structural data of inactive m-calpain is reported in PDB and the authors suggested

the mechanism of the activation and a conformational change for the active-site formation.

2P-029 真核生物の翻訳開始因子 eIF5B の構造解析

Structural analysis of eukaryotic translation initiation factor eIF5B

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Eukaryotic protein synthesis consists of three processes which are initiation, elongation and termination. In these processes, initiation is the most complex and strictly regulated. To initiate protein synthesis, the small ribosomal subunit with bound initiator methionyl-tRNA is first assembled at the start codon of an mRNA, and then binds to the large ribosomal subunit, leading to start the elongation process. This process is not only required the coordinated activities of ribosome, initial tRNA and mRNA, but also at least 12 translation initiation factors in eukaryotes (eIFs).

The protein eIF5B, a prokaryotic homolog of IF2, is one of the few translation initiation factors conserved in all organisms. It is a ribosome-dependent GTPase and promotes the ribosomal subunits joining that is final step in initiation process and required for the interaction between eIF5B and another initiation factor, eIF1A (prokaryotic homolog of IF1). Such interaction between eIF5B and eIF1A plays an important role in translation initiation. To clarify the final step of initiation process, the structural analyses of these proteins are necessary.

In this study, recombinant *S. cerevisiae* eIF5B and Se-Met substituted eIF5B were cloned, expressed, purified and crystallized successfully. Subsequently, a Se-SAD dataset of 2.9 angstrom resolution was collected at SPring-8 BL41XU and initial phases were obtained with SHELX and SOLVE/RESOLVE.

In this presentation, we will discuss the results of the structural analysis of eIF5B.

2P-030 Structural modelling of human Aryl hydrocarbon receptor and QM/MM study of its interaction with dioxins

Structural modelling of human Aryl hydrocarbon receptor and QM/MM study of its interaction with dioxins

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Xenobiotics such as dioxin binds to Aryl hydrocarbon receptor (AhR) with an extremely high affinity leading to a complex sequence of events that causes the toxicological and biological effects of dioxin. It is believed that the toxicities of xenobiotics depend strongly upon their receptor affinities. We investigate this crucial interaction between the dioxin and the AhR by using structural modelling, molecular docking simulations, and QM/MM calculations, to understand the toxicity of dioxins. Since the 3D structure of the human AhR has not been determined so far, we construct a structural model of its ligand binding domain for the first time by applying homology modelling techniques. The modelled structure was then evaluated thoroughly by using different validation methods. The obtained structure was then subjected to molecular dynamics simulations with explicit solvent water molecules. In the calculation, the modelled structure was found to be remarkably stable for approximately 2 ns after the early relaxation phase. Next, we predict the most probable sites of interaction between dioxin and AhR by applying a molecular docking strategy previously developed by us. Then, we perform QM/MM calculations by using our newly developed interface program connecting QM and MM engines. This study identifies the relationship between the structure and toxicities of the xenobiotics.

2P-031 放射光を用いた S-SAD 法によるネイティブタンパク質結晶構造解析

Structure determination of a native protein crystal by S-SAD phasing with synchrotron radiation

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S-SAD phasing is the method to determine a crystal structure using a lower energy X-ray and weak anomalous signals from light elements such as sulfur that are naturally present in almost all proteins. Because it requires only a native protein crystal for phasing, this phasing method has great advantages for high-throughput protein crystallography and for the structure determination of membrane proteins of higher organism, which are quite difficult to be produced as SeMet-incorporated variants. While S-SAD phasing has such great advantages, there are some experimental difficulties. For example, a lower energy X-ray exhibits stronger anomalous signals from light elements, but also exhibits higher absorption effect which might deteriorate data qualities and leads unsuccessful experiments. In order to overcome these difficulties and facilitate this phasing method, further developments have been performed at the Photon Factory. BL-17A is a short gap undulator beamline and a highly brilliant X-ray beam at 6 ~ 8 keV is available. In S-SAD phasing, such a brilliant X-ray beam is very efficient and

In this presentation, such application and recent development of BL-17A will be reported.