identifiable neurons. Nonetheless, despite the continued attention given to the physiological characteristics of its nervous system, the genetic information of the *Lymnaea* central nervous system (CNS) has not yet been fully explored. The absence of genetic information is a large disadvantage for transcriptome sequencing because it makes transcriptome assembly difficult. We here performed transcriptome sequencing for *Lymnaea* CNS using an Illumina Genome Analyzer IIx platform and obtained 81.9 M of 100 base pair (bp) single end reads. For *de novo* assembly, five programs were used: ABySS, Velvet, OASES, Trinity and Rnnotator. Based on a comparison of the assemblies, we chose the Rnnotator dataset for the following blast searches and gene ontology analyses. The present dataset, 116,355 contigs of *Lymnaea* transcriptome shotgun assembly (TSA), contained longer sequences and was much larger compared to the previously reported *Lymnaea* expression sequence tag (EST) established by classical Sanger sequencing. The TSA sequences were subjected to blast analyses against several protein databases and other molluscan EST data. As a result, the richness of the present TSA data allowed us to identify a large number of new transcripts in *Lymnaea* and molluscan species.

1E1400 分子動力学シミュレーションによる Hsp90 と ADP の結合自由エネルギープロファイル

Free energy profile for binding of ADP to Hsp90 with molecular dynamics simulation

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Binding of a ligand molecule to a protein plays a key role in the function of many proteins. The functional cycle of proteins is driven by the binding of some of the ligand molecules, and is inhibited by the binding of some of the other ligand molecules. Hsp90 (Heat Shock Protein 90) is one of a group of molecular chaperones required for protein folding, maturation and trafficking of client proteins. Recent studies have shown that Hsp90 is associated with many pathologies: cancer, virus infectious diseases, protein folding disorders and neurological diseases. Hsp90 is an attractive target for the therapies of many pathologies. The functional cycle of Hsp90 is driven by ATP binding and hydrolysis to ADP. However, the processes of binding and hydrolysis of ATP and dissociation of ADP are not revealed at atomic resolution. The purpose of our study is to elucidate the free energy profile for binding of ADP to Hsp90. We performed all-atom molecular dynamics (MD) simulations of Hsp90 with ADP. The initial structure used in our simulations is the X-ray crystallographic structure of the N-terminal domain of human Hsp90 in complex with ADP (PDB code: 1byq). We calculated the free energy profile for binding of ADP to Hsp90 using the thermodynamic integration method. We will report the results of the free energy calculation.

1E1412 シャペロン空洞内の変性タンパク質が受けるコンフォメーション制限

The conformational restriction of denatured protein encapsulated in the chaperonin cage

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Chaperonin assists protein folding in its closed cage. Recently, in addition to the isolation of protein into the cage to reduce a risk of aggregation, it has been proposed that the folding rate of denatured protein is accelerated by the restriction of conformational space in the cage. Hartl's group suggested that the formation of disulfide-bond in the slow folding mutant of maltose binding protein (DMMBP) resulting in more rapid folding as wild-type MBP mimics the conformational restriction in the chaperonin cage. However, on the contrary, our recent results showed that denatured protein encapsulated in the cage is not completely isolated and can even escape out of the cage.

In the last meeting, we reported that blue fluorescence protein starts its folding from more unfolded state than its spontaneous folding, using FRET to observe its conformational change. Here, we applied same technique to maltose binding protein (MBP) and its slow folding mutant (DMMBP). If Hartl's notion is true, the distance between cysteine residues of MBP that used for disulfide-bond formation should be smaller than that of DMMBP. However, their distance changes during folding are almost same. Therefore, the folding acceleration of DMMBP in the chaperonin cage is not caused by disulfide-bond-like conformational restriction, and thus our proposition of unfolding effect in the cage is more probable. We will report the comprehensive study of the conformational effect in the chaperonin cage on MBP and DMMBP at this session.

1E1424 小角 X 線散乱によるアミロイド線維形成の初期会合プロセスの解析 Investigation of initial assembling process of fibrillation by small angle X-ray scattering

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Amyloid fibrils are supramolecular assemblies associated with many degenerative diseases including Alzheimer's, prion, and Huntington's diseases. It has been proposed that several smaller aggregates such as oligomers and protofibrils are involved in the fibrillation process, but little is known about a detailed picture how these species contribute to form the mature amyloid fibrils with a rigid cross- β structure. To explore this issue, we have performed time-resolved small angle X-ray scattering (SAXS) measurements of insulin fibrillation combined with analyses of thioflavin T fluorescence and secondary structure. The SAXS data were monitored every 9 seconds immediately after the initiation of fibrillation reaction inside a quartz cell.

As a result, we have found that insulin molecules transform into a characteristic oligomeric structure in the very early stage of lag phase. After the accumulation of oligomers, the scattering curves further increased, implying the assembling process of oligomers into mature fibrils. Interestingly, a distinct type of oligomers in terms of size, cross- β content, and thioflavin T fluorescence intensity was observed when fibrillation reaction was performed at a high salt concentration, suggesting the presence of diverse oligomeric structures that may contribute to a variety of fibrillation pathways and furthermore, the manifestation of polymorphism of mature amyloid fibrils.

1E1436 Distinguishing crystal-like amyloid fibrils and glass-like amorphous aggregates from their kinetics of formation

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Amyloid fibrils and amorphous aggregates are two types of aberrant aggregates associated with protein misfolding diseases. Although they differ in morphology, the two forms are often treated indiscriminately. β 2-Microglobulin (β 2m), a protein responsible for dialysis-related amyloidosis, forms amyloid fibrils or amorphous aggregates depending on the NaCl concentration at pH 2.5. We compared the kinetics of their formation, which was monitored by measuring thioflavin T (ThT) fluorescence, light scattering, and 8-anilino-1-naphthalenesulfonate (ANS) fluorescence. ThT fluorescence specifically monitors amyloid fibrillation, whereas light scattering and ANS fluorescence monitor both amyloid fibrillation and amorphous aggregation. The amyloid fibrils formed via a nucleation-dependent mechanism in a supersaturated solution, analogous to crystallization. The lag phase of fibrillation was reduced upon agitation with stirring or ultrasonic irradiation, and disappeared by seeding with preformed fibrils. In contrast, the glass-like amorphous aggregates formed rapidly without a lag phase. Neither agitation nor stirring accelerated the amorphous aggregation. Thus, by monitoring the kinetics, we can distinguish between crystal-like amyloid fibrils and glass-like amorphous aggregates. Solubility and supersaturation will be key factors for further understanding the aberrant aggregation of proteins.

1E1448 細胞濃度に近い無添加細胞抽出液の調製

A method to prepare cell extract near intracellular concentration without additional salts and buffers

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For the aim to elucidate essential processes for synthesizing cells, we have established a method to prepare additive-free cell extract having high concentration like cells. At first, essentiality of buffers and salts for preparing functional cell-free translation extract was verified. Exponential growth cells of *Escherichia coli* were washed with double distilled water (DDW) at twice, and were dissolved with DDW. After freezing, the cells were disrupted by sonication. S30 fraction of the obtained cell extract (DDW-S30) showed sufficient activity for cell-free protein production of GFP and luciferase. Next, a gradual evaporating method to accumulate biological components has been established. Here, we used vacuum desiccator to accumulate samples. Total volume decreased 20 μ l per hour on average, and 4.5-fold concentration of BSA was obtained thorough the methods. The methods also achieved