obtained from the trajectory of molecular dynamics simulation of calmodulin and the scattering intensities were calculated from the structures. In this calculation, observed intensity changes could be explained by the averaged intensities of the fluctuated structures.

1G1348 AAA ファミリー蛋白質 p97 の ATP 依存的な構造変化の解析

Observation and characterization of dynamic ATP-dependent movements of the p97 AAA ATPase using high-speed atomic force microscopy

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p97 is an evolutionarily conserved AAA (ATPases associated with diverse cellular activities) family chaperone. p97 forms a double (D1 and D2) ring-shaped homo-hexamer, which is a characteristic of type II AAA proteins, and catalyzes conformational changes of substrate proteins upon ATP hydrolysis. The noticeable feature of p97 is its involvement in a variety of cellular processes such as endoplasmic reticulum-associated protein degradation and other protein quality control activities. Despite accumulating knowledge on the multiple cellular functions of p97, details of molecular mechanisms of the protein remodeling by p97 and binding modes of cofactor/adaptor proteins to p97 are largely unknown.

In this study, we have observed and analyzed ATP-dependent movements of p97 on a sub-second time scale using high-speed atomic force microscopy (HS-AFM). The HS-AFM observation of p97 revealed the hexameric ring structure with a central hole, and the height and diameter of p97 were closely similar to those of the crystal structure. In the presence of ATP, the D1 ring of p97 rotated clockwise relative to the D2 ring, which was fixed to the surface of mica plate. The D1 ring of p97 stayed in the rotated state for ~0.2 sec on average, and returned to the original position. ADP did not induce this rotation. To know the mechanism of the rotational movement in more detail, we are analyzing movements of p97 mutants, which have lost ATP binding or ATP hydrolysis ability, and those in the presence of non-hydrolyzable ATP analogues.

1G1412 細菌べん毛フック繊維連結部の極低温電子顕微鏡による構造解析

Structural analysis of the flagellar hook-filament junction by electron cryomicroscopy

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The bacterial flagellum is a biological nanomachine for the locomotion of bacteria. The flagellum consists of three functional parts: the basal body, which acts as a rotary motor; the filament, which acts as a helical propeller; and the hook, which acts as a universal joint. The hook and filament, which consist of FlgE and FlgC, respectively, have mechanically distinct characteristics, as the former is flexible and highly curved while the latter is rather rigid with a large radius of curvature. The hook-filament junction is made of two proteins, FlgK and FlgL, which are responsible for connecting these two mechanically distinct structures. In general, the hook-filament junction works as a mechanical adapter, the mechanism of which is still unknown. To reveal this mechanism, we analyzed the structure of the junction by using electron cryomicroscopy (cryoEM). We used a mutant strain in which the expression level of flgC can be controlled by tetracycline to make the filament length relatively short. This made image data collection highly efficient. We also developed a new method of image analysis for the hook-filament junction. We obtained a 3D density map, which shows the shape and array of subunits in the junction structure. And we report a partial atomic model of the hook-filament junction obtained by molecular model of FlgK, FlgL, FlgC, FlgE. This will advance our understanding of the hook-filament junction, which will allow for adaptor mechanism.

1G1424 冷陰極電界放出銃を用いた、クライオ電子顕微鏡法による小さい生体分子複合体構造解析への挑戦

Challenges to elucidate small biomolecular complex structure in electron cryo-microscope with a cold field emission gun

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Electron cryo-microscopy (cEM) is one of the powerful techniques to observe protein structure without staining. However, since the method gives us low contrast images, to date, it has been used to analyze protein complexes with above 100kDa, e.g., virus and ribosome. Unfortunately, we cannot easily observe a typical size of proteins (approximately 30 kDa, less than 100 kDa). This is why this method usage is limited. In the present studies, we challenged observation of small proteins by cEM and their 3D reconstruction to expand the possibilities of cEM.

We here observed both porcine pepsin (34.6kDa) in gastric juice and porcine pepsinogen (39.6kDa), pepsin zymogens, as test samples. Since electron micrographs are low in contrast, it is difficult to observe these small proteins. Thus, we used cEM with a cold field emission gun and an energy-filtering device (EF-2000), to resolve the problem. In addition, we examined effects of noise reduction filters and thickness of the ice layer to know optimum conditions for observing small proteins.

As a result, we succeeded in observing both proteins directly and reconstructing 3D structures at about 2 nm resolutions. When we compared both 3D reconstructions, we could certainly observe that pepsin has a cleft near an active site, while pepsinogen has no clear cleft. Comparing them with their crystal structures, loop regions of crystal structures were found to be different in structure. Thus we certainly showed that cEM can be applied to even typical small proteins.

1G1436 X線溶液散乱強度を用いたタンパク質の構造変化の推定

Estimation of protein structural change by using X-ray solution scattering intensity

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The function of a protein is closely related with the folding structure, so elucidation of the structure becomes an important question in understanding the function. X-ray solution scattering technique is useful in molecular structural study because of the little limitation in making the sample and its possibility of experimenting under a physiological condition, but the atomic structure cannot be uniquely determined due to the low resolution. In this study, an effective method was constructed to derive the three-dimensional structure of the protein promptly from the X-ray solution scattering intensity. The optimal structure was presumed by changing the initial structure to fit its scattering intensity to the target scattering intensity. The initial structure was chosen from already-known structure of different chemical state. Changing the structure was based on a coarse-grained molecular dynamics. The parameters of the force field were chosen to reflect the conformational information of the initial structure. As a result of applying this simulation to calmodulin, the structure under the condition of calcium ion existence was able to be presumed from the X-ray solution scattering intensity of it and the structure under the calcium ion non-existence. The field of molecular structural study using X-ray solution scattering technique is expected to extend by this study being applied to more proteins.

1G1448 SAXS 法によるタンパク質立体構造の計算科学的解析

P12 Computational analysis of protein structure by small-angle X-ray scattering

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The major bottleneck in the structure determination and study of dynamics of biopolymers is that their molecular weights are extremely higher than those of other molecules.

Small-angle X-ray scattering (SAXS) has no upper limit in molecular weights, and structural information from SAXS is enough to construct the low resolution models by *ab initio* modeling, therefore SAXS is suitable to reduce the number of degrees of freedom in the system and very effective against the above-mentioned problem.

We previously developed a new program, SAXS_MD, which is based on the restrained molecular dynamics algorithm and refines the protein structure with SAXS data. In this study we performed the structural calculation by restrained molecular dynamics with both NMR and SAXS constraints and investigated the additivity and complementarity between them in order to elucidate the essential structural information that defines the protein architecture. RNase T1 is used as a model protein, which has already been determined in NMR method unambiguously.

As the result of the structural calculation with SAXS data and intentionally extracted NMR distance information, it is indicated that SAXS information complements the tertiary structural information from NMR of moderate distance