1P022 SPring-8 における微小タンパク質結晶構造解析の現状
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Present Status of Protein Micro-crystallography at SPring-8
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BL41XU is an undulator beamline at Japanese third-generation synchrotron facility SPring-8. This beamline has been improved for obtaining high quality data from protein micro crystals (~ 25 μm) using a micro beam (~ 25 μm). The new K/B mirror system was installed at the autumn of 2006, and the monochromatized beam is focused to V70 x H100 μm (F.W.H.M.) at sample position. The new diffractometer was also installed in May, 2007. The final beam size at sample position is defined from 25 x 25 to 70 x 100 μm²(F.W.H.M.) by using two set of quadrant slits. Photon flux and flux density of the 25 x 25 μm² beam at 1 Å are 3.0 x 10¹¹ photons/sec and 4.8 x 10¹⁴ photons/sec/mm², respectively. It is essential for the data collection to control the radiation dose since micro crystals will be received remarkable radiation damage. The data collection software installed to BL41XU can change the beam irradiation point automatically to suppress the effect of radiation damage by using multiple positions on a crystal and multiple crystals. Data collections of the structure-unknown samples with the crystal size from 15 to 50 μm were performed by using this tool, and we successfully obtain the initial structure by SAD phasing at the resolution from 2.3 to 3.9 Å. We present the current status of protein micro-crystallography at BL41XU.

1P024 海鼠 (グミ) 由来溶血性レクチン CEL-III の多量化機構の X 線小角散乱による解析
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1aE01 Analysis of the oligomerization mechanism of hemolytic lectin CEL-III derived from sea cucumber by small-angle X-ray scattering
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CEL-III is a Ca²⁺-dependent, Gal/GalNAc-specific lectin purified from the sea cucumber *Cucumaria echinata*, which shows haemolytic activity by oligomerization on the cell surface. We found that the oligomerization of this protein can be induced not only in lipid membranes but also in solution under the appropriate conditions. CEL-III forms an oligomer in solution when complexed with lactose at high pH values and in the presence of high concentrations of salt. To elucidate the oligomeric structure and mechanism of oligomerization of CEL-III, small-angle x-ray scattering (SAXS) measurements were carried out. Previous report shows that the molecular weight of the oligomer is determined as 1019 k from its forward scattering value by SAXS which is much larger than that estimated using SDS-PAGE, 270 k. To elucidate the effects of surfactants, we measured SAXS in the presence of the surfactants. The results showed that the oligomer dissociated into hexamer (290 k), which well agreed with the result of SDS-PAGE. Kratky plot of both profiles showed that the two peaks which are typical for oligomeric proteins, α-hemolysine, one of the pore forming proteins associates into heptamer on the cell surface. These results suggest that the oligomeric structure in the presence of the surfactants is a minimum unit of hemolytic activity and oligomer without surfactant consists of 4 oligomers with surfactant by weak interactions.

1P023 リアルタイム X 線小角散乱でみたシアノバクテリア時計蛋白質の離合集散ダイナミクス
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1pB01 Real-time SAXS Observation of Assembly and Disassembly Dynamics of Cyanobacterial Circadian Clock Proteins
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Incubation of three clock proteins, KaiA, KaiB, and KaiC, in the presence of ATP induces a KaiC phosphorylation cycle that is a potential clock pacemaker in cyanobacterium *Synechococcus elongatus* PCC 7942. To elucidate oscillatory mechanisms, we followed the oscillatory dynamics of the Kai proteins using real-time small-angle x-ray scattering techniques.

The scattering pattern of a ternary mixture robustly oscillated with a period of approximately 24 h, indicating a repeated assembly and disassembly of Kai complexes. Interestingly, apparent forward scattering intensity was phase delayed relative to the KaiC phosphorylation cycle. Quantitative SAXS measurements of the ternary and binary mixtures revealed that the phase of the *in vitro* oscillation is mainly determined in early stages; where the assembly and disassembly dynamics is phase delayed because of kinetically preferred KaiA/KaiC interactions followed by thermodynamically preferred KaiB/KaiC interactions. In the presentation, we will discuss the size and shape of these clock complexes.

1P025 立体構造変化に伴い機能発現する抗菌ペプチドの設計
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1aE02 Designing an antimicrobial peptide whose function is induced upon structural transformation
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Proteins have functions in the native state and lose them quickly when denatured. By making use of this property, we try to design a protein whose structure and activity can be transformed reversibly. Antimicrobial peptides suppress growth of bacteria and/or kill them, and are usually produced in a living body of human and other species. Because of these facts, antimicrobial peptides attract our attention as a new antibiotic having a low toxicity. In this study, we try to design an antimicrobial peptide that obtains its function only in particular environment, such as high/low pH caused by bacterial infection. Because such a peptide excludes only the invading bacteria, they can be used as a good therapeutic compound. In this study, we focus on a tryptophan residue (Trp), which has a large hydrophobic sidechain, and elucidate a relationship between the accessible surface area (ASA) of Trp sidechain and the activity to judge whether we can design an antimicrobial peptide whose function is induced by a change in the ASA of a Trp upon structural transformation. Referring to bovine milk protein, *Lactoferrin*, we designed 6 peptides whose first Trp in the sequences lies in a different amino-acid context so as to have the different sidechain ASA. We determined the solution structures using NMR, and measured antimicrobial activity by MIC method. A correlation between the sidechain ASA of the Trp and the activities was found, indicating that we can control antimicrobial activity by changing the ASA of a Trp.