the inhibitors restrict the steric requirement at the residue at the P1' position. In addition, active site residues at the S1' position are advantageous for Pro at the P2' position.

1P012 カルボニル還元酵素 S1 の基質認識機構の構造学的解析 Structural analysis for substrate recognition of carbonyl reductase S1

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Chiral compounds are important building blocks for medicines and becoming more important for pharmaceutical industries. Carbonyl reductase S1 from *Candida magnoliae* (S1) catalyzes ethyl-4-chloro-3-oxobutanoate to optically active ethyl (S)-4-chloro-3-hydroxybutanoate with high optical purity of 99.9% enantiomeric excess, which is an important chiral building block of HMG-CoA reductase inhibitor. To reveal the structural basis of the high-level stereoselectivity of S1, we determined the crystal structure of S1 in complex with NADPH at a resolution of 2.0 Å. The structure of S1 shows that S1 belongs to the short chain dehydrogenase/reductase superfamily and forms homotetramer in the crystal. Based on this structure, we constructed the substrate-binding model of S1. The substrate-binding model of S1 shows that the high-level stereoselectivity is caused by the shape of a substrate binding pocket and a substrate recognition by residues including Ser178 and Trp239.

1P013 X 線小角散乱法による分裂酵母 Swi5-Sfr1 の溶液構造解析 Solution structure of S.pombe Swi5-Sfr1 studied by small-angle X-ray scattering

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The Swi5 and Sfr1 proteins in the fission yeast *Schizosaccharomyces pombe* are mediators of DNA homologous recombination. The Swi5-Sfr1 complex simulates Rhp51-dependent DNA strand exchange that is a central reaction of homologous recombination. Despite its importance, a three-dimensional structure of the Swi5-Sfr1 complex has not been determined yet. Therefore, we measured small-angle X-ray scattering (SAXS) of Swi5, Swi5-Sfr1, Swi5-Sfr1ΔN177.

The SAXS results indicate that Swi5 forms a tetramer in solution, and has an elongated shape. In the Swi5-Sfr1 complex, two Swi5 molecules are bound to one Sfr1 molecule, and the length of the Swi5-Sfr1 complex is longer than that of a Swi5 tetramer, and the length of Swi5-Sfr1ΔN177 complex is approximately half of the Swi5-Sfr1 complex.

However, due to low resolution of SAXS structures, it is unable to determine locations of the N- terminal and intermediate regions of Sfr1 in the complex. Thus, by using the Fab of the IgG antibody specifically binding to the N- terminal and intermediate regions of Sfr1, we attempt to determine the locations of these domains in the complex. Currently, we identified the epitope of Sfr1 by a Western blot and measured SAXS for the Swi5-Sfr1-Fab complex.

The SAXS results indicate that N-terminus of Sfr1 in the complex located the end of the elongated shape, and the intermediate region of Sfr1 in the complex is located at the center of the elongated shape.

1P014 ストップドフロー X 線小角散乱測定による溶血性レクチンの構造変化の解析 Analysis of the structural change of the hemolytic lectin by stopped-flow 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 structural change in oligomerization of CEL-III, stopped-flow 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. In the presence of detergents, oligomer dissociated into hexamer (290 k) and the radius of

gyration of oligomer was 62 to 75 Å, which well agreed with the result of SDS-PAGE. The results of stopped-flow small-angle x-ray scattering showed that the oligomerization process occurred milli second order under the high pH condition. This is the first report about the dynamic structural change measurement in oligomerization process of CEL-III.

1P015 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 FlgI, 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 (junction) is made of two proteins, FlgK and FlgL, which are responsible for connecting these two mechanically distinct structures. In general, the 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 flkC 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 junction. In this method, we use 1D-projection alignment and a proper reference by using the helical symmetries of the hook and filament, respectively. We obtained a 3D density map at 25 Å resolution, which shows the shape and array of subunits in the junction structure. However, the resolution is still insufficient to elucidate the connection mechanism. Further improvements in cryoEM imaging and image analysis are under way.

1P016 Biophysical characterization of highly active recombinant *Gaussia* luciferase expressed in *Escherichia coli*

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Bioluminescence, the enzymatic generation of visible light, is widely observed in nature including firefly and marine copepods. Recently, the smallest bioluminescent protein (MW: 19.9kDa), *Gaussia* luciferase (GLuc), has been isolated from the marine copepod *Gaussia princeps* and has attracted much attention as a reporter protein. However, preparation of large quantities of homogeneous natively folded recombinant GLuc appears to be difficult due to its ten cysteines. This has hampered both the biophysical characterization and engineering of GLuc, limiting its use in bioluminescence imaging.

Here, we report the biophysical characterization of recombinant GLuc expressed using a novel *Escherichia coli* expression system based on a cold induced expression vector (pCold). Using this system, a large fraction of the protein was expressed in the soluble fraction. GLuc, purified exclusively from the supernatant using nickel affinity chromatography, yielded a large amount of pure GLuc with a native disulfide bond pattern (Soluble-GLuc). Soluble-GLuc had a strong bioluminescence activity and it retained 65% of its activity after 30 minutes incubation at 95°C. Soluble-GLuc remained fully folded until 40°C, as assessed by circular dichroism; and the thermal denaturation curve was S-shaped, indicating a cooperative transition, with a midpoint temperature of 56°C. These results indicate that both the structure and bioluminescence activity of GLuc remain stable at high temperatures, and they strongly suggest GLuc's potential as a reporter protein.

1P017 Trp-Cys 距離に基づくスタフィロコッカスヌクレアーゼの変性状態の観測 Urea denaturation of staphylococcal nuclease monitored by tryptophan-cysteine distance

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Denatured structure of proteins has been less understood than their native structure because crystallographic studies give the structure of folded proteins. Some experiments have revealed that proteins take residual structure even in denaturants. Characterization of the residual structure has a great impact to many problems concerning proteins such as folding, the mechanism of which can change by considering the residual structure, not a random coil. Staphylococcal nuclease (SNase) is good to be studied because the folding is affected by mutation, which may modulate the residual structure. We have