metabolism of lipoproteins and their uptake in tissues. Some mutations in apolipoproteins are related to inheritable disease. In this work, we studied physical properties of amino acid mutations in two groups: mutations with and without disease susceptibility in apolipoproteins. The analysis was carried out by two steps: First, we enumerated peaks of MR-index plots from amino acid sequences, and the mutations which changed the position of the MR-index peak were used for further analysis. Second, we calculated the average hydrophobicity and charge density of seven residues, and changes in the magnitude as well as the position of MR-index peaks. MR-index is an index that estimates allergen-specific amino acid sequence and binding site. The result indicated that the properties were significantly different between the mutations with and without disease susceptibility. The results will be useful for the prediction of inheritable diseases of multi-factors.

2PT109 L-リシン酸化酵素のX線結晶構造解析

Structure of L-lysine α -oxidase from *Trichoderma viride*

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L-amino acid oxidase (LAO) is a dimeric flavoenzyme that catalyzes oxidative deamination of an L-amino acid to produce a 2-oxo acid with ammonia and hydrogen peroxide. Most of known LAOs show wide substrate specificity, but almost no activity for L-lysine oxidation. Unlike other LAOs, L-lysine α -oxidase (LysOX) from *Trichoderma viride*, which is found during the search for antitumor agents, exhibits strict substrate specificity for L-lysine. Thus, LysOX is an attractive candidate for an enzyme-based amino acid sensor. Moreover, LysOX has broad antitumor activity. To understand the strict substrate recognition mechanism of LysOX, structural information is essential. Here we report the crystal structure of LysOX from *T. viride* at 1.9 Å resolution. Since no active enzyme was expressed in *E. coli*, we purified LysOX from wheat bran culture of *T. viride*, and used it for crystallization. The diffraction data were collected at Spring-8 beamline BL41XU and the structure was determined by molecular replacement using the structure of L-amino acid oxidase from *Vipera ammodytes* as a search model. LysOX is composed of three domains and forms a tetramer in the crystal. The structural refinement is now in progress.

2PT110 Differences between key amino acid residues for the transitions L- to R- and R- to L- protofilaments of Salmonella flagellar filament

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Salmonella cell swims by rotating its flagellar filaments. The filament (~10 μ m) is composed of ~30,000 molecules of a single protein flagellin and forms left-handed helical structure under physiological conditions. The cell repeats swim-and-tumble and consequently move toward their favorite environments and away from unfavorites. The swim-and-tumble is caused by the change of the filament shape introduced by reversal of flagellar rotation, and the change of the filament shape is called polymorphic transformation. To elucidate the transformation mechanisms of flagellar filaments, we applied genetic suppressor analysis within flagellin molecule against SJW1655 producing R-type straight filament and SJW1660, HFG180, and HFG195 producing L-type straight filament. We isolated about 100 revertants from every mutant, determined the second mutation sites, investigated the localization of the amino acid residues on L- and R-type flagellin molecules, measured their swimming and swarming speeds, and observed their flagellar filaments. From these results, we propose that S106, A416, A427, and R431 are key residues involved in transitions from L- to R-type protofilaments, whereas D107 and G426, and S448 are keys from R- to L-type protofilaments. From the differences in the key residues, we suggest that L to R (R to L) transition is not just the inverse reaction of R to L (L to R) transition.

2PT111 アミロイドペプチドのオリゴマー形成メカニズムに関する構造学的解析

Structural analysis of the oligomerization mechanism of amyloid beta peptide

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Amyloid beta peptides (A β) form senile plaques following oligomerization in the brain. A β oligomers are thought to be neurotoxic and to play a key role in Alzheimer's disease (AD). A β 40, 42 and 43 are major species of A β . It is known that A β 42 and 43 are more prone to aggregation than 40. Thus, the length of the C-terminus is assumed to promote A β oligomerization and neurotoxicity. This study aims to investigate the structure of A β oligomers and the oligomerization mechanism associated with AD.

To examine the influence of the C-terminus on A β properties, we compared the oligomerization propensity of A β by PICUP (Photo-Induced Cross-linking of Unmodified Proteins). All species formed dimers, however, large oligomers (trimers and tetramers) were formed at higher rate by A β 42 and 43. Further, NMR analyses suggested that the C-terminal structure of A β 42, 43 differs from that of 40. Besides, the flexibility of the C-terminus was higher in A β 40 than in 42 and 43 revealed by NMR. The results showed that the interaction between the C-terminus of A β 42 and 43 is stronger than that of 40.

To investigate the cross-linking sites, we used ESI-TOF MS to analyze for tryptic decomposition product of A β dimer stabilized by PICUP. It was found that H6-K16 fragments were cross-linked. In A β dimers, this region may exist in close proximity to the corresponding region on the other molecule.

In conclusion, our data suggested that A β forms parallel dimers. Moreover, a difference in C-terminal structure is important in the formation of large oligomers.

2PT112 Role of the Tyr residue stacked on the si-face of the isoalloxazine ring moiety in ferredoxin-NADP⁺ oxidoreductase from *Bacillus subtilis*

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Ferredoxin-NADP⁺ oxidoreductase (FNR, [EC 1.18.1.2], [EC 1.18.1.3]) from the low-GC gram-positive bacterium *Bacillus subtilis* (BsFNR) is similar to bacterial thioredoxin reductases (TDRs) in amino acid sequence. The crystal structure analysis of BsFNR revealed the presence of the aromatic residue Tyr50 stacked on the si-face of the isoalloxazine ring of the FAD prosthetic group within a distance of approximately 3.5 Å. This conformation has been often found among the flavoprotein super family including FNR from plastid, but TDRs lack such the residue. To investigate the role of Tyr50 in catalysis with NADPH and ferredoxin (Fd), we performed site specific mutation analysis. While Tyr50Trp mutant retained reactivities with NADPH and Fd of ~20 and ~40%, against those of the wild type respectively, replacements to nonaromatic Gly and Ser residues drastically decreased the reactivities with both NADPH and Fd.

2PT113 アミロイド構造の決定におけるタンパク質揺らぎの役割の解析 NMR study on the role of protein fluctuation in determining the amyloid conformation

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Aggregation-prone proteins often misfold into multiple distinct amyloid conformations. Although amyloid conformation is crucial factor to determine its toxicity or infectivity, the formation mechanism of diverse conformation remains unclear. Yeast prion Sup35, the [PSI⁺] protein determinant, forms several types of amyloid with different conformations. Sup35 is translation termination factor composed of three domains (N, M, and EF). An EF is globular domain carrying translation termination function. N and M are intrinsically disordered domains required for amyloid formation, however, biological function is unknown. In the present study, we elucidate the role of protein fluctuation in determining the amyloid conformation using NMR spectroscopy. We found that the Sup35-NM mutant, S17R, dramatically altered the amyloid core region. The S17R-type amyloid conformation can propagate to wild-type Sup35-NM easily. These results raised an intriguing possibility that there are (more than) two non-overlapping amyloid core regions in the Sup35-NM sequence and one of the regions is selected depending on the condition under which Sup35-NM amyloid is formed. NMR analysis with CLEANEX-PM and STD revealed that the amino acid residues in both amyloid core regions showed fast exchange rates and their transient interactions in the oligomers might act as a nucleus for amyloid growth.

2PT114 抗真菌活性をもつ DUF26 タンパク質 Gnk2 の構造と機能 Structure and function of Gnk2, a DUF26 protein with antifungal activity