1P067 酵母プリオン Sup35 の細胞内 1 粒子解析**Single Particle Tracking of Yeast Prion Sup35 in Living Cells**

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Yeast prion Sup35 forms self-propagating fibrillar aggregates. The mechanism of yeast prion propagation consists of fibril growth, division, and transmission to daughter cells. To unravel how individual prion particles propagate and transmit to the daughter cell, here we have conducted single particle tracking of Sup35-GFP aggregates in living yeast cells using highly inclined thin light. Since the prion particles were abundant in a cell, we had to reduce the number of the foci. Treated with guanidine hydrochloride, which specifically inhibits Hsp104, we succeeded in tracking the distinct particles in the cytoplasm, and the mean square displacement analysis showed that most prion particles underwent Brownian motion.

1P070 SEP タグを用いたタンパク質凝集の時系列的解析**Analysis of protein aggregation kinetics using short amino acid peptide tags**

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We report the effects of 10 representative amino acids on the aggregation kinetics of proteins measured as the solubility of a simplified BPTI variant, to which short artificial tags containing the amino acid of interest were added at its C-terminus. We determined the solubility of the tagged variants as a function of equilibration time (20 minutes to 48 hours) and total protein concentration (0.10 mg/ml to 25.0 mg/ml). We rationalized our observations by identifying three different solubility values: A “Transient Solubility (TS)”, an “Aggregation Initiation Concentration (AIC)” and a “Long-term Solubility (LS)”. TS was dependent on the measurement conditions, but AIC and LS can be considered as amino acid intrinsic properties.

1P068 高圧 Native PAGE 法によるリゾチーム変異体が形成するアミロイド原繊維のかい離過程の定量的解析**Quantitative Analysis of High Pressure Native PAGE on Dissociation of Lysozyme Variant Amyloid Protofibril**

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A disulfide-deficient variant of hen lysozyme, OSS, is known to form an amyloid protofibril spontaneously, and to dissociate into monomer under high hydrostatic pressure. We carried out native PAGE at various pressures and temperatures, to characterize the dissociation equilibrium of OSS amyloid protofibrils. The obtained electrophoresis patterns were composed of the leading band corresponding to the monomeric OSS and the protofibrils stacked on the top of gel media. The amount of monomeric OSS increased cooperatively with pressure, and none of the intermediates emerged. These observations were quantified by densitometry, and analyzed based on the theoretical model, to reveal the volumetric parameters of amyloid protofibril.

1P071 短いテトラペプチドの全原子シミュレーションによるアミノ酸の無定形な凝集性の洞察**All atom molecular dynamics simulation of short tetra-peptides shed insights into amino acid's amorphous aggregation propensities**

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In this study, we aim at relating experimentally measured amino acid aggregation propensities to values estimated from 100ns all atom molecular dynamics (MD) simulations. The experimental amino acids aggregation propensities were determined as the soluble fraction upon centrifugation of our model protein (BPTI-19A) to which a 5-residue tag made from a single amino acid type was fused. The simulation was carried out using AMBER 8 on a MD Grape special purpose computer for a 104Å cubic box filled with >30,000 water molecules and containing 27 peptides made of a single amino acid type. We performed the analysis for 14 types of representative amino acids. Overall, our simulation was able to reproduce the relative solubilities of all residues.

1P069 リゾチームジスルフィド欠損変異体の線維化反応の温度依存性**Temperature-Dependence of Fibrillation of Lysozyme Disulfide-Deficient Variant**

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Hen lysozyme disulfide-deficient variant OSS, which is intrinsically unfolded, spontaneously forms amyloid-like fibrils in a strongly salt-concentration dependent manner. Here we studied temperature-dependence of the OSS fibrillation by monitoring the progress of polymer formation primarily with size-exclusion chromatography. The fibrillation rate generally became lower with increasing temperature indicating that some reaction step with equilibrium of negative temperature-dependence is involved. The observation that the elution profile of the fibrils formed at lower temperatures showed a higher fraction of smaller fibrils, and therefore a higher number concentration of polymers, suggests that the nucleation of fibrils has the negative equilibrium temperature-dependence.

1P072 リボアミド脱水素酵素のフィードバック制御機構**The feedback regulation mechanism of dihydrolipoamide dehydrogenase**

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The feedback regulation mechanism displayed by Dihydrolipoamide dehydrogenase (E₃) was confirmed through the steady state and time-resolved fluorescence analysis of FAD included in E₃. The enzymatic activity analysis suggested that K_m for dihydrolipoamide (DHLip), one substrate of E₃ was reduced by NAD⁺ which was the other substrate. When the fluorescence of E₃ was measured in the presence of DHLip, it was rather suppressed by NAD⁺ to suggest that the conversion of FAD to FADH₂ by DHLip would be accelerated by the binding of NAD⁺ with E₃. The time-resolved fluorescence of FAD and Trp in E₃ demonstrated that the space around FAD would be so extended by the binding of NAD⁺. The conformational change that DHLip could approach more easily to FAD is induced by NAD⁺ binding.