It is experimentally known that the heat-denaturation temperature of a protein $T_m$ is raised by the sugar addition. The magnitude of this effect stabilizing the protein depends on the sugar species [1]. In earlier work, we proposed a measure of the thermal stability of a protein, which is defined as the solvent-entropy gain at 298 K upon protein folding $S$ normalized by the number of residues [2,3]. $S$ was calculated using a hybrid of the angle-dependent integral equation theory combined with the multipolar water model and the morphometric approach. Here we show that $S$ can be calculated using the hard-sphere solvent whose number density and molecular diameter are set at those of water. We then investigate the effects of sugar addition on the thermal stability by considering water-sugar solution modeled as a binary mixture of hard spheres. The three-dimensional integral equation theory is employed. The thermal stability is determined by a complex interplay of the molecular size of the sugar $D$ and the total packing fraction of the solution $\alpha$. $D$ is estimated from the volume per molecule in the sugar crystal, and $\alpha$ is calculated using the experimental data of the solution density. We find that the thermal stability is considerably enhanced by addition of sucrose. In the presentation, we will discuss the effects of addition of not only sugars other than sucrose but also osmolytes.


1PT138 ヒトヒアルピロイド誘導体形成に影響する因子の解析
Amyloid fibril inhibition mechanism of human calcitonin
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Human calcitonin (hCT) is a 32-residue peptide hormone. hCT is used as a medicine for osteoporosis, but it easily forms amyloid fibrils under physiological condition, which is a problem on medication. Salmon calcitonin (sCT) is known to inhibit amyloid formation of hCT. Combinational use of hCT and sCT is expected to improve the pharmacological activity. While the N-terminal region (9-19) of sCT takes an $\alpha$-helix, the C-terminal half of the helix is disordered in hCT. Although an interaction between the disordered region and the C-terminal tail of hCT is proposed to play a role on the amyloid formation, the detailed mechanism of the inhibition of the amyloid formation by sCT has not been revealed. In this study, we investigated amyloid fibril formation of hCT in the presence of each of two chimeric peptides, which are composed of the 1-13residues of hCT and the 16-32residues of sCT and, the reverse combination. The former region (1-15) contains the stable $\alpha$-helix and the latter the flexible region in hCT. Amyloid fibril formation was monitored by fluorescence intensity from Thioflavin T. While the fluorescence intensity raised about 30-40 hours after incubation of hCT alone, the amyloid fibril formation was inhibited in the presence of sCT, or each of the two chimeric peptides, indicating that each of the N-terminal and the C-terminal regions of sCT possesses the inhibitory ability. From these results, we conclude that both the C-terminal region and the N-terminal stable $\alpha$-helix are engaged in the inhibition of the amyloid fibril formation.

Analysis of Almost Reversible Conformational Change of Amyloid Fibrils by Double pH-jump
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To obtain insights into the mechanism of amyloid fibril conversion, pH-jump experiments were performed using a H2 peptide of mouse prion protein. Previously, we reported that H2 peptide formed ordered amyloid fibrils with an immensely large minimum at 207 nm on CD spectrum at pH 2.9 (named pH2 fibrils), but aggregate-like fibrils with a minimum at 220 nm were formed at pH 7.5 (named pH7.5 fibrils) near its isoelectric point. In this study, firstly single pH-jump from 2.9 to 7.5 was performed. As a result, the CD spectrum showed that the pH2.9 fibrils instantly changed to pH7.5-like fibrils with a minimum at 218 nm, but the ellipticities were certainly distinct between pH7.5-like and pH7.5 fibrils, implying that the conformation of pH2.9 fibrils partially remains even at pH 7.5. Moreover, the pH7.5-like fibrils almost returned to the pH2.9 fibrils by restoring the solution pH from 7.5 to 2.9. FT-IR spectra indicated that these conformational changes were caused by the disruption of highly ordered $\beta$-sheet and $\beta$-turn conformation, and the subsequent their reconstructions. In addition, kinetic conformational changes of the fibrils after the single and double pH-jumps were examined using ANS fluorescence stopped-flow. As a result, these conversions of the fibrils composed of a few phases were accomplished within several seconds. Thus, amyloid fibrils can be changed readily between the distinct conformations separated by a low energy barrier almost reversibly.

1PT140 金属結合によるプロテオグリカン-バクサンベプチド繊維の構造変化
Conformation change in peptide fragments of human prion protein caused by metal binding
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Prion diseases are transmissible spongiform encephalopathies (TSE) and lethal infectious diseases caused by misfolding of prion protein (PrP). Human prion protein (huPrP) binds metal ions at the residues 60-91 (His61, His69, His77, His85) and residues 90-126 (His96, His111) in N-terminal. Histidine residue are known as high-affinity sites to metal binding. Metal binding to PrP is considered as an important process for misfolding of PrP. This report aims to investigate conformation change in huPrP caused by metal binding. huPrP Peptide fragments containing his96 and his111 were examined for binding metal such as Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$. $\Delta$Pr values were determined with electro titrimetric analysis (Metrohm, 840 titrino plus). Metal binding to peptide fragment was observed using visible absorption spectra (Hitachi, U-2800 Spectrophotometer) and Circular Dichroism (CD) (JASCO,J-820). Secondary structure of peptide fragment was observed using Far-UV CD. Addition of Cu$^{2+}$ and Ni$^{2+}$ to huPrP (93-102:GTTGHSSQWN) showed the shift of $\Delta$Pr value. Vis absorption and Vis CD indicated the characteristic spectra resulting from metal binding. It was derived from imidazole N bound to metal ion and from d-d transition of metal ion. Far-UV CD spectrum showed the conformational change of secondary structure induced by metal binding. We will also investigate metal binding to peptide fragments of huPrP containing His111 by using UV/Vis spectroscopy, CD and electron spin resonance (ESR) to further understand about metal binding to huPrP.

1PT141 ユピキチンの温度-圧力-自由エネルギー地形図
Temperature-pressure-free energy landscape of ubiquitin
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To understand the change of packing and/or solution of protein denaturation from quantitative point, volume change $\Delta V$ and isothermal compressibility change $\Delta \kappa_T$, and thermal expansion coefficient change $\Delta \alpha$ are needed. These thermodynamic parameters at given temperature and pressure are determined from temperature($T$)- pressure($p$)- free energy change($\Delta G$) landscape, which is obtained from a combined series of pressure/temperature variable denaturation experiments.
In this study, we explore the ($T, p, \Delta G$) landscape of ubiquitin. FTIR spectra of ubiquitin was reversibly changed by temperature/pressure perturbation at $p=2.0$ and $p=5.5$. To determined $\Delta G$ at given temperature and pressure, temperature/pressure transition curves were obtained from change in FTIR peak intensity at 1673 cm$^{-1}$ which is assigned to native $\beta$-strand structure. Three-dimensional ($T, p, \Delta G$) landscapes and thermodynamic parameters ($\Delta V$, $\Delta T$, $\alpha$, entropy change $\Delta S$ and heat capacity change $\Delta C_p$) at ambient temperature and pressure (298K, 0.1MPa) were obtained.

1PT142 X線結晶構造解析におけるファジアル系センサの応用: 形態制御
Ferritin assembly kinetics followed by small-angle X-ray scattering