(HNA-1), and the other is an oxidation product higher than mixed disulfide (HNA-2). We have developed a convenient HPLC system to separate HSA into HMA and HNA. To examine the redox state of cerebrospinal fluid (CSF), we analyzed the percentage of reduced and oxidized albumin fractions in human lumbar CSF together with serum from 42 patients with orthopedic operation. They were divided into two groups by age (young group, average age = 29.1 years ($n = 10$); older group, 65.4 years ($n = 32$)). For albumin in serum, mean values for the fractions of HMA, HNA-1 and HNA-2 were 76.4, 22.0 and 1.6% in the young group, and 69.5, 28.6 and 1.9% in the older group, respectively. In contrast, for CSF, those values were 93.0, 6.7 and 0.3% in the young group, and 93.1, 5.8 and 1.1% in the older group, respectively: there was an extremely large amount of HMA fraction as well as a markedly small amount of HNA fraction in both groups. From our HPLC results, significant high value for the SH titer of albumin molecules in CSF in both groups indicates that the redox state in brain is maintained in highly reduced status.

2TA1-02 Kinase Inhibition modeling by Proteometrics and docking analysis

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We report the recognition of stable and unstable kinase-inhibitor complexes by Proteochemometrics, a ligand-target modeling approach, using Support Vector Machines (SVM) classifiers. The algorithm consists of the development of topological auto-correlation descriptors for protein-inhibitor complexes, and then extracting their key features using Principal Component Analysis (PCA). An SVM model is then developed to relate the PCA-derived feature vectors to the stability class (stable or unstable) of protein-inhibitor complex. Use of autocorrelation features was compared with fragment-based approach and the former was found to outperform the latter. Final classifier could recognize 83% of data to be stable or unstable using jackknife type of validation and test-set prediction. Analysis of substructure classification showed a very homogeneous behavior on the whole target-ligand space. Finally, kinase binding mode diversity was analyzed by docking the active ligands against six different kinases, and candidates for multi-kinase inhibition were identified by averaging highest ranked docking scores among all the kinases targets. The kinase inhibition predictor is available online at: <http://gibk21.bse.kyutech.ac.jp/llamosa/KinasePCA-SVM/KinasePCA-SVM.html>.

2TA1-03 プロテアソームによる蛋白質分解における最適なユビキチンシグナルと分解開始部位の距離

Optimal spacing between ubiquitin modification and unstructured initiation site for efficient proteasome-mediated degradation

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The eukaryotic 26S proteasome controls diverse cellular processes by degrading specific regulatory proteins. Most proteins are targeted for degradation by two elements: a covalently attached polyubiquitin tag for binding to the proteasome, and an unstructured region that serves as the initiation site for proteasomal proteolysis. Here, using substrates with polyubiquitin (Ub₄) chain and ubiquitin-like (Ubl) domain, we have characterized how the length and the location of the unstructured initiation site affect degradation. We found that a model substrate is degraded efficiently only when its initiation site has a certain minimal length and is properly separated from ubiquitin modification in space. These length and spacing requirements for degradation are different between substrates with Ub₄ chain and Ubl domain. Presumably, short unstructured regions, regions too close to or far from the ubiquitin modification are inaccessible to the proteasome. Thus they cannot induce degradation. These results suggest that the length and location of the proteasome initiation site affect a protein's fate and that they play a central role in selecting proteins for proteasome-mediated degradation.

2TA1-04 高圧力 NMR による di-ubiquitin の構造揺らぎ

Conformational fluctuation of di-ubiquitin studied by variable pressure NMR spectroscopy

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Conformational fluctuation of K48-linked di-ubiquitin (di-Ub) has been studied by variable pressure NMR technique. Here, to characterize the dynamics of the proximal-Ub (selectively ¹⁵N labeled), which is connected to Gly 76 of distal-Ub at its Lys 48 side-chain, ¹⁵N spin relaxation NMR measurements are carried out at 65 and 2650 bar. The R1 and NOE indicate that rapid segmental motions of the backbone in the pico-to-nano second range are similar at two pressures. In contrast, larger R2 values are clearly observed at higher pressure at residues in b2 and b5 strands and main α -helix. The increased R2 could originate primarily from

the fluctuation between conformers N1 and N2, as previously observed in mono-Ub. In addition, preferential decrease in HSQC cross-peak intensity is noted for several residues at the C-terminal side of the protein at 3030 bar, indicating the presence of an intermediate conformer, with local disorder. This conformer nearly coincides with the locally disordered conformer I previously identified in mono-Ub. The results indicate that the proximal-Ub shares a common feature in the conformational fluctuation with mono-Ub. In addition, differences in pressure response of ¹H and ¹⁵N chemical shifts are noted between mono-Ub and the proximal-Ub. We will also report pressure-induced changes in the mutual orientation of the proximal and distal Ubs.

2TA1-05 シャペロニンは変性タンパク質の一部に結合しつつフォールディングを援助する

Chaperonin assists the folding of denatured polypeptide partly tethered on GroEL

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In the chaperonin-assisted protein folding, it is widely accepted that upon GroES binding to the GroEL-substrate polypeptide complex, the polypeptide is immediately released into the cage and starts folding. We found, on the contrary, that polypeptide is bound to GroEL apical surface and partially protrudes from the cage to be dragged out by antibody from outside. The release of bound polypeptide from GroEL to inside the cage is the rate-limiting step for chaperonin assisted folding. The released polypeptide completes its folding very quickly. The fact that artificially cross-linked rhodanese folds to the folding competent intermediate indicates that polypeptide release is concomitant with intermediate formation. The folding competent intermediate folds rapidly in the cage, but it folds slowly out of the cage. This suggests that the competent intermediate formed in the cage is different from that formed in spontaneous folding. We concluded that the hydrophobic interaction between GroEL apical surface and denatured polypeptide in the chaperonin cage enables protein folding with avoiding irreversible kinetic trapped state and changes the folding pathway from that of spontaneous folding.

2TA1-06 タウ由来ペプチドの繊維性凝集におけるリン酸化の部位的效果

Positional effects of phosphorylation upon fibrillation of tau derived peptide

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Phosphorylation of a fibrillogenic protein, human tau, is believed to play crucial roles in pathogenesis of the Alzheimer's disease. For elucidating molecular mechanisms of the phosphorylation effect upon tau fibrillation, we synthesized short peptides derived from partial sequences surrounding tyrosine or serine residues, which are plausible in vivo phosphorylation sites in the fibril forming domain of tau. Those model peptides form amyloid-type fibrils similar to those generated by full-length tau. A variety of mutated peptides carrying the phosphotyrosine or phosphoserine residues were synthesized, and their fibrillation processes were studied systematically. The results showed that the phosphorylation of any sites significantly influenced the fibrillation of the peptides. However, the effects were dependent critically upon the location of the phosphate group in the peptide sequence. Based on the observations, we suggested that interaction of the phosphate group with the neighboring sites generate a variety mode of interaction depending on chemical properties of the neighboring molecular components. The intra- and inter-molecular modes of the interactions alter not only the thermodynamic and kinetic properties of the fibrillation but also the nano-scale morphology of the fibrils. A computer simulation analysis utilizing crystal coordinates of the fibrils was also performed for interpreting the experimental results. Results obtained by experimental and simulation techniques are to be presented in the meeting.

2TA1-07 変性剤が水分子の分極におよぼす効果を考慮した、タンパク質フォールディングのシナリオ: 低濃度の HDO をプローブとする IR 法による研究

A scenario for folding of proteins in terms of the changes in the polarization of the water induced by denaturants; b-Lactoglobulin in aqueous 2,2,2-trifluoroethanol

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We carried out measuring FTIR spectra of HDO as the probe of the water in aqueous 2,2,2-trifluoroethanol (TFE) solutions of b-lactoglobulin (BLG). We studied changes in the frequencies of $\nu(\text{O}-\text{D})$ and $\nu(\text{H}-\text{O}-\text{D})$ vibration bands with the concentration of TFE in the absence and the presence of BLG. We also studied the conformational changes in BLG by observing the changes in the Amide-I and Amide-II bands of it with the concentration of TFE.

In both the solutions of TFE without and with BLG, $\nu(\text{O}-\text{D})$ and $\nu(\text{H}-\text{O}-\text{D})$ shifted to the higher frequency side and to the lower one, respectively. This shows