atmospheric pressure using a vibrating tube digital density meter (Anton Paar DMA 60/605). From experimental density data, the partial molar volumes of proteins were estimated. It was found that the partial molar volume increases for myoglobin, decreases for BSA, and is constant for lysozyme with increasing C6OH molarity. Similarly, the partial molar volumes were obtained for the TFE systems. From these results, the volume and binding behavior of proteins and alcohols was examined in relation to the hydrophobicity of protein surface and the rigidity of protein structure.

3P-002 高温での溶液 NMR 測定における蛋白質の NDSB による安定化

Stabilization of proteins by NDSB for solution NMR measurements at elevated temperatures

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In solution NMR, better sensitivity and resolution are obtained by raising sample temperature, but proteins aggregate at a too high temperature due to thermal denaturation. Because pharmaceutically important proteins tend to aggregate more easily than housekeeping proteins, prevention of protein aggregation is one of challenging tasks in protein NMR. Here we report the application of NDSB (non-detergent sulfobetaine) for protein NMR. NDSB enables acquisition of protein NMR spectra at elevated temperatures by stabilizing proteins. Sample precipitation and time-dependent decrease in NMR signals due to higher temperature were prevented by supplementing NDSB in protein samples. NDSB is useful because this compound does NOT 1) increase viscosity appreciably, 2) increase pulse length in contrast to regular salts, 3) perturb static and dynamic structure of proteins, nor 4) interfere with regular protein-protein or protein-ligand interactions. Strong noises originating from NDSB, which are observed in 1H-13C planes, are eliminated by using a deuterated NDSB analog we synthesized. This NDSB analog is also useful because it exhibits lower viscosity than commercially available NDSB (about half). Multidimensional multinuclear measurements regularly employed for protein structure determinations can be performed in the presence of this new compound, and we report the structure of proteins determined in its presence. We will also report general precautions that help to prevent aggregation of proteins and peptides occurring during NMR measurements.

3P-003 モルテングロビュール状の変性状態においてピロリドンカルボキシルペプチダーゼ (PCP) の折りたたみ反応は順次進行していく

The folding of PCP (Pyrrolidone Carboxyl Peptidase) sequentially proceeds in the denatured state similar to the stage of molten globule

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Our research interest is to visualize the processes of PCP folding, but it seemed to be difficult because the folding is an all-or-none transition. That is, almost all cross peaks on HSQC spectra originated in the native state simultaneously appeared with an identical rate constant upon the PCP folding. However, we recently found that the conformations of denatured PCP after removal of 6 M GuHCl at 4°C and pH 3.0 (we call D1-state) sequentially changed from a flexible state to more rigid ones against the amide H/D exchange during the incubation. After the incubation of PCP in the D1 state for a desired time at 4°C and pH 3.0, H/D exchange reaction was carried out, and then partially deuterated PCP was refolded by increasing the temperature and their HSQC spectra were measured. Just after the removal of 6M GuHCl (transfer to D1 state), only α 6-helix was strongly protected against the H/D exchange. After 3 hours incubation in the D1 state, α 3-, α 4-helices and a part of α 2-helix were protected besides α 6-helix. After 3 days incubation, the regions of β 1, α 1, β 2, β 3, β 4 and β 9 were further protected, although changes in HSQC spectra in the D1 state were not observed. These results suggest the progress of PCP folding under the D1 state similar to the molten globule stage. It is likely that the final conformation in the D1 state is very close to the native one, but exactly not equal to the native one. The cooperative folding transition to the native state should occur after that.

3P-004 スタフィロコッカスクレアーゼの分子内構造へのカルシウムイオン効果の1分子力学測定

Single-molecule force-measurement of calcium ion effects on intramolecular structure of staphylococcal nuclease

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To detect the calcium ion effect on staphylococcal nuclease (SNase), we have performed quasi-static mechanical unfolding of SNase both in the absence of Ca^{2+} ion (-Ca SNase) and in the presence of Ca^{2+} ion (+Ca SNase) using intermolecular force microscopy, modified AFM with high resolution. Single protein molecules were extended with a constant velocity by controlling the end-to-end distance. Force-extension curves of both -Ca SNase and +Ca SNase were composed of one or two peaks at various distances. The distribution of end-to-end distance of the peaks showed 6 peaks, indicating multiple intermediates. The peak locations of -Ca SNase were similar to those of +Ca SNase, but the unfolding peaks appeared with different frequencies.

To characterize the calcium ion effect, we performed clustering analysis of all force-extension curves. The force-extension curves was clustered into 10 clusters. Force peaks with higher frequencies in the absence of Ca^{2+} ion appeared at the breaks of α -domain, and those in the presence of Ca^{2+} ion appeared at the breaks of β -barrel domain. As unfolding peaks corresponded to the ruggedness of energy landscape, the results indicate that the ruggedness of energy landscape of β -barrel domain increased but that of α -domain decreased in the presence of Ca^{2+} ion. This change reflects that the Ca^{2+} ion binding induces the stabilization of β -barrel domain.

3P-005 IgG C_H3 ドメインの安定性に対する分子内ジスルフィド結合の影響の研究

The effect of the intermolecular disulfide bond in IgG C_H3 domain to its stability.

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One of antibody molecules, IgG, consists of 12 immunoglobulin domains, two of which are C_H3 domains. The C_H3 domain has one intramolecular disulfide (SS) bond. In an IgG molecule, two heavy chains are linked by two intermolecular SS bonds at the hinge region and non-covalent interaction at the C_H3 domains. As the affinity of the homodimerization of the C_H3 domain is very strong, C_H3 is assumed to stabilize the antibody molecule. Some recent reports shows that the intramolecular SS bond of C_H3 of monoclonal antibody is occasionally reduced. It is suggested that the breakage of the SS bond can cause a destabilization of C_H3 domain moiety, leading to subsequent decreases in stability and activity of the whole antibody. The purpose of the present study is to clarify the contribution of the SS bond to the C_H3 structure and stability in the residue level.

We chose NMR experiment as a main method to monitor the conformational change of C_H3. Prior to NMR measurements, we established a C_H3 expression system using *Pichia pastoris*. With this system, we achieved a yield of about 100 mg C_H3 from one-liter broth, which is quite higher yield than that using *E. coli*. After the uniform $^{13}\text{C}/^{15}\text{N}$ labelling of C_H3, we achieved 90% of signal assignments of the main chain atoms.

We will show the conformational change caused by the reduction of the SS bond and discuss its contribution to the stability and folding process of the C_H3 molecule on the basis of the results of the NMR, CD, fluorescence, and guanidine-hydrochloride titration experiments.

3P-006 高温における牛血清アルブミンの構造変化に対する界面活性剤の添加効果

Additive Effect of Surfactant on the Structural Change of Bovine Serum Albumin at High Temperatures

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The additive effect of sodium dodecyl sulfate (SDS) on the structure of bovine serum albumin (BSA) in the thermal denaturation was examined by means of CD measurement. The helicity (66%) of the protein decreased with rise of temperature. When the BSA solution was cooled down to 25 °C after heating at temperatures above 50 °C, the helicity was not completely recovered. The higher the thermal denaturation temperature was, the lower the recovered helicity was upon cooling down. On the other hand, upon the addition of SDS, the secondary structure of BSA was partially protected against the thermal denaturation above 50 °C where the structural change became irreversible. The disruption of the protein structure was restrained upon the coexistence of SDS of extremely low concentrations. For example, the helicity was 42% at 70 °C in the absence of SDS, but it was maintained at 59% at the same temperature upon the coexistence of 0.50mM SDS. Upon cooling down from 70 to 25 °C, the helicity of BSA was recovered up to about 60% in the presence of SDS of the same concentration. The protective effect of SDS is characterized by the specific amphiphilic nature of this anionic surfactant. This is considered to be attained by building cross linking bridges of the surfactant ion between particular nonpolar residues and particular positively-charged residues in the protein molecule.

3P-007 β -ラクトグロブリンの α -ヘリックス中間体の寄与の解明

A study of contributions of the α -helix intermediate of β -Lactoglobulin to its folding process.

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We investigated contributions of the α -helix intermediate of β -lactoglobulin to its folding process. In this study, we aimed to have knowledge about a relationship between stability of intermediate and folding rate. So, we did this study by using some mutants, helix propensity of whose intermediates are changed.

Prepared three mutants (C121A, C121A/G17E, C121A/E44L). C121A inhibits shuffle between disulfide bonds and free cysteine group. Thus, we consider C121A as a pseudo-wild type. From the result of time course measurements, α -helix content of intermediates were obtained. From the results, we found that intermediate of C121A/G17E is more α -helix-rich and that C121A/E44L is less than that of C121A, respectively.

Furthermore, we revealed that C121A/E44L acquires new intermediate in folding process but transition energies of intermediates are not changed. From the results, it is so modification of the burst-phase intermediate changes whole folding pathway. Thus, formation of α -rich burst-phase intermediate is requisite