1P-070 アポブラストシアニン折りたたみの熱力学：理論と実験との定量的比較

Thermodynamics of apoplastocyanin folding: Comparison between experimental and theoretical results.

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It has been shown experimentally that the folding of apoplastocyanin accompanies a very large enthalpic loss [1]. This implies that an even larger entropic gain occurs in stabilizing the folded structure to overcome the enthalpic loss. Here we calculate the water-entropy gain upon the folding of apoPC using the angle-dependent integral equation theory combined with the multipolar water model and the recently developed morphometric approach [2]. It is demonstrated that the calculated value is in quantitatively good accord with the value estimated from the experimental data by accounting for the conformational-entropy loss. According to a conventional view [3], the water adjacent to a hydrophobic group is unstable especially in terms of the rotational entropy and the folding is driven primarily by the release of such unfavorable water to the bulk through the burial of nonpolar side chains. We show, however, that the resultant entropic gain is too small to elucidate the experimental result. The great entropic gain observed is ascribed to the reduction of the restriction for the translational motion of water molecules in the whole system.[1] N. Baden, S. Hirota, T. Takabe, N. Funasaki, and M. Terazima, J. Chem. Phys. 127, 175103 (2007).[2] T. Yoshidome, M. Kinoshita, S. Hirota, N. Baden and M. Terazima, J. Chem. Phys. 128, 225104 (2008).[3] W. Kauzmann, Adv. Protein Chem. 14, 1 (1959).

1P-071 ミオシン・ロッドにおけるスキップ残基の役割 — 合成ペプチドを用いた検討

Role of skip residues in myosin rod as revealed by synthetic peptide studies

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Myosins from striated muscle of cephalopods consist of two heavy chains and four light chains. We have sequenced the myosin heavy chains from four species of cephalopods by cDNA cloning, and found that all of them have very similar structures to those of mammalian striated muscle. Above all, four skip residues were conserved in the rod regions, whose coiled-coil structures are considered to be disturbed by the presence of these residues. In the present study, attempts were made to examine the role of these skip residues by using several synthetic peptides. Peptides (30mer) were synthesized by Fmoc method based on the partial sequence of myosin heavy chain of Japanese common squid *Todarodes pacificus*, namely, W1343 to A1372 (WSKFESEGAARAD E LYDSKRKLQAELESEA) containing four heptad repeats. The purified peptides were dissolved in 0.1 M KCl, 50 mM phosphate (pH 7.0), 1 mM dithiothreitol, 0.1% sodium azide, and were subjected to spectropolarimetry in the temperature range of 4-60°C. The temperature-dependency of α -helical content was then calculated. When the center residue, glutamic acid, was deleted, no significant change in the helical content was observed. However, when the residue was replaced by glutamine, glutamine or isoleucine, the helical contents of the peptides were greatly reduced together with their thermostability, suggesting that the side chain of skip residue could greatly affect the coiled-coil structure of myosin rod. Physiological role of the partially loosened rod portion is to be investigated.

1P-072 3つのSS結合をもつヒルジンの酸化リフォールディング経路の解析

Analysis of oxidative refolding pathways of hirudin with three disulfide bonds

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In our laboratory, we have recently succeeded in observation of the slow SS rearrangement reactions separately from the SS formation reactions in the oxidative refolding of ribonuclease A by using 3,4-dihydroxyselenolane oxide (DHS^{ox}). In this paper, this highly effective oxidizing reagent was applied for the analysis of oxidative folding pathways of hirudin, which has three SS bonds in the native state. The folding reaction of a hirudin variant (CX-397) was initiated by addition of DHS^{ox} to the reduced form (R) under various conditions. According to the HPLC analysis, it was revealed that the oxidation reaction (i.e., SS formation) completed quantitatively within 1 minute, producing the 1S, 2S and 3S intermediates containing 1, 2, and 3 SS bonds, respectively. After the initial oxidation reaction was finished, the native structure (N) was gradually regenerated at pH 10.0. At the same time, slight formation of a conformationally stable 2S intermediate (2S^{*}) was observed. This intermediate would be formed from the 2S intermediate ensemble by the SS rearrangement reaction and could be oxidized to N. At pH 8.5, generation of N was not clearly observed, but when L-Cys was added to the reaction solution, N was regenerated in a good yield from the 3S intermediate ensemble through the intermolecular SS exchange reactions. It was, therefore, suggested that hirudin has at least two distinct folding pathways; one is going through the 2S^{*} intermediate, and the other is a direct pathway from 3S to N via the intermolecular SS exchange reactions.

1P-073 Congo red のラマン散乱をプローブとする細胞膜表面におけるアミロイド線維形成の追跡

Raman scattering from Congo red as a probe of amyloid fibril formation on the cell membrane

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The neuronal plasma membrane is considered to serve as a platform for assembly of amyloid β -peptide (A β) into neurotoxic fibrils. Although Raman microspectroscopy is a potential tool for monitoring the fibril formation of A β on the surface of cell membranes, direct observation of Raman signals from the fibril is difficult because of the interference from other membrane and cellular components. In this study, we have examined the utility of a histological dye for amyloid fibrils as a resonance Raman probe of the fibril formation of A β on cell membranes.

The direct dye Congo red (CR) is known to have a high affinity for amyloid fibrils. In the 488 nm-excited resonance Raman spectrum of CR, strong bands assignable to the N=N stretching vibrations of the azo group are observed at 1405 and 1380 cm⁻¹. Upon binding to A β fibrils, the intensity ratio I₁₃₈₀/I₁₄₀₅ was increased from about 1 to 1.3. In contrast, neither β -sheet-rich globular proteins nor lipid membranes affected I₁₃₈₀/I₁₄₀₅ of CR, indicating that the intensity ratio may be used as a marker for detection of fibrils. We also examined micro-Raman spectra of CR-stained A β fibrils on a single cell-sized liposome composed of phosphatidylcholine, cholesterol, sphingomyelin, and gangliosides. The resonance Raman bands of CR at 1405 and 1380 cm⁻¹ could be observed without serious interference from lipids or the peptide. Resonance Raman microspectroscopy of CR may be useful in monitoring the fibril formation of A β on neuronal cell membranes.

1P-074 粗視化モデルを用いた全原子シミュレーションの効率化

Sampling enhancement for all-atom simulation using coarse-grained model

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Proteins are complex systems characterized by rugged free-energy landscapes having many local minima. Such minima are separated by high free-energy barriers, and thus, conventional all-atom model (AAM) simulations suffer from a problem known as kinetic trapping. One way to overcome the problem is coarse-grained model (CGM) simulation, and it enables extensive conformation sampling. Then, we proposed a method which combines AAM for accuracy and CGM for efficiency. In our method, a simplified model in which residues are represented as single beads centered in their Ca positions is used as CGM. We have tested the sampling method on a protein-G. First, the conformation space was searched roughly using CGM, and then, many structural samples around the folded, transition and unfolded states were obtained from the CGM simulation. Since these samples have simplified structural representation, they were reconstructed with full atomic details to be used as initial structures of AAM simulations. Each reconstructed system was energy minimized and heated from 1K to 300K using the program, presto. Then, the conformation ensemble with full atomic resolution at room temperature was obtained on a wide phase space. We discuss the efficiency of our method comparing it with the conventional sampling methods.

1P-075 新規インスリンフィラメントの構造解析と生化学特性

Structural Properties and Cytotoxicity of Novel Insulin 'Noodle'-like Filaments

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Insulin is a small peptide hormone that is known to form amyloid fibrils under acidic conditions. We have previously shown that filamentous ('noodle'-like) insulin amyloid which was different from fibrous ('needle'-like) insulin amyloid was produced under a mild reductive condition using tris (2-carboxyethyl) phosphine hydrochloride (TCEP). The CD spectra showed that both of insulin fibrils and filaments contain a β -sheet structure. Nevertheless, thioflavin T (ThT) binding property was very different between them, suggesting a difference in inner structure. Importantly, MTT assay using rat PC12 cell line showed that the filaments were not cytotoxic although the fibrils were toxic. In this study, we examined their cell toxicities more in detail using other cell lines, and also examined their inner structures using novel luminescent conjugated polyelectrolyte probes (LCPs).

The cytotoxicity of the insulin filaments against human HEK293 cell line was also extremely low while the fibrils were toxic, suggesting that the insulin filaments were generally nontoxic. The fluorescence measurement in the presence of Polythiophene acetic acid (PTAA), one of the LCPs, showed that PTAA weakly bound to the insulin filaments and that the insulin fibrils' spectrum revealed a spectral red shift in comparison to the PTAA-filaments interaction. This suggests that the insulin 'noodle'-like filaments are formed by loose assembly of insulin molecules. More detailed studies on cytotoxicity and inner structures are in progress.

1P-076 筋タンパクのメカニカルアンフォールディングに対する温度の影響

Influence of temperature on mechanical unfolding of muscle proteins

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The mechanical stability of proteins is important for their function. Atomic force microscopy (AFM) is one of the useful techniques for studying this at the single molecule level. In this study we have investigated the influence of temperature on mechanical unfolding of I27 domain of human cardiac titin and tail domain of