3P061 天然変性蛋白質の立体構造特性に関わるリン酸化の静電的な制御**Phosphorylation as an electrostatic regulation of the conformational state of intrinsically disordered proteins**

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An intrinsically disordered protein (IDP) often exhibits folding when binding to its partner, which is frequently regulated by phosphorylation; e.g., KID (kinase inducible domain) binds to its partner protein upon phosphorylation and adopts an α -helix. One of the effects of phosphorylation should be the electrostatic perturbation on the intra-molecular interaction which affects the conformational state of IDP; in fact, phosphorylated KID (pKID) exhibits higher helix content than KID. We here studied the conformational change of IDPs upon phosphorylation using a side-chain-coarse-grained model that can reproduce the increase of helical content for pKID, and suggest the switching mechanism between inter and intra-molecular electrostatic interactions.

3P062 ウマ β ラクトグロブリン初期中間体における非天然ヘリックスのフォールディングキネティクスへの影響**Effect of non-native α -helix in the early intermediate on folding kinetics of equine β -lactoglobulin**

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Equine β -lactoglobulin is a small globular protein (162 residues) which is monomeric at any pH. Although ELG adopts a predominantly β -sheet structure consisting of nine anti-parallel β -strands (A-I) and one major α -helix in the native state, it has a markedly high helical propensity throughout the sequence. Previously, we have shown that a non-native α -helical intermediate accumulates in the early folding stage of ELG and that the region corresponding to the H strand assumes a non-native α -helix. To investigate the contribution of non-native α -helix on refolding kinetic of ELG, we construct a mutant in which helical propensity at the H strand region is decreased and then the refolding kinetics were investigated by the stopped-flow CD and fluorescence.

3P063 天然条件下における PCP 各残基アンフォールディング速度の観測—尿素によるアンフォールディングの促進機構**Observation of unfolding rates of each residue of PCP under the native condition - Mechanism for urea to accelerate the unfolding rate**

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To study the unfolding process under the native condition, H/D exchange reactions were measured every residue. H/D exchange reactions with the slowest rate followed the EX1 mechanism, reflecting the unfolding rate of individual residues. The structural fluctuations in the native state are extremely restricted, and water penetration into the interior of protein was prohibited. The influence of urea was examined on the structural fluctuations in the native state. Urea had no influence on the regions easily exposed to water, but had strong influence on the interior of protein into which water penetration is prohibited. We will discuss the mechanism for urea to accelerate the unfolding rate.

3P064 高速溶液混合法を用いたアポミオグロビンの salt-induced 中間体のフォールディングに関する研究**Folding of salt-induced intermediate of apomyoglobin using ultrarapid mixing methods**

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Proteins consisted of more than 100 amino acid residues accumulate folding intermediate during the folding process. Some proteins accumulate equilibrium intermediate under moderately denaturing conditions, which have similar properties to the folding intermediate. An additional intermediate is formed under acid denaturing conditions in the presence of high concentration of salt (salt-induced intermediate). In this study, folding mechanisms of salt-induced and equilibrium intermediate of horse apomyoglobin are compared in terms of the equilibrium and kinetic properties of these intermediates by means of fluorescence measurement using ultrarapid mixing methods.

3P065 変異体解析を用いた緑色蛍光蛋白質のフォールディング機構におけるヒスチジン残基の役割に関する研究**The role of histidine residues in folding mechanism of green fluorescent protein studied by mutagenesis approach**

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Green fluorescent protein (GFP) accumulates several intermediates under moderately acidic conditions. A previous study suggested that GFP accumulated a native-like equilibrium intermediate (N') with reduced green fluorescence at pH 5, which arose from histidine residues with abnormal pK_a values.

In this study, to examine the role of histidine residues in unfolding of GFP, a series of variants was constructed by replacing histidines with other amino acids and the acid-induced equilibrium unfolding measurements were carried out by monitoring the chromophore fluorescence. From these results, the contribution of each histidine residues on the pH-dependent stability of GFP will be discussed.

3P066 スタフィロコッカ・ヌクレアーゼの安定性とフォールディング/アンフォールディングの研究**The Stability and Folding/Unfolding of Staphylococcal Nuclease at the Residue Level**

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we used native-state hydrogen-deuterium (H/D) exchange NMR to estimate the stability of staphylococcal nuclease at the residue level, and replica exchange molecular dynamics simulations to monitor the secondary structure formation/unfolding on temperature-induced unfolding. These results suggest that the more stable regions (β -barrel domain) under the native conditions unfold at the higher temperatures. In addition, the pattern of the stability obtained the native-state H/D exchange NMR experiments is associated with that of the protection factors at 100 ms after the initiation of the folding obtained by the previous H/D exchange pulse-labeled NMR study. Our results indicate the relationship between proteins' stability and conformation change in protein folding.