2P084 C末端欠損スタフィロコッカルスクレアーゼが示す誘導折り畳み反応の分子メカニズム
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Molecular mechanism of induced-folding of C-terminal truncated staphylococcal nuclease
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We have shown that the inhibitor induced-folding of Δ140-149 Staphylococcal nuclease proceeds through the formation of inhibitor-conjugated intermediate (US complex). In order to understand the molecular mechanism of the formation of the US complex, it is essential to elucidate the binding site at the US complex. In this study, we prepared several Ala substituted mutants of Δ140-149 at the substrate binding sites in native conformation and at the sites far from the binding sites. The difference binding free energy between the Ala mutants and Δ140-149 was estimated by titration experiments of the US and FS formation. V51A/Δ140-149 where V51 is distant from the binding site showed no remarkable free energy changes both in FS and US. In contrast, those of (T82A, K84A, Y85A, Y113A, V114A, or Y115A)/Δ140-149 were highly affected to decrease about 1 kcal/mol of the binding free energy both in FS and US. These sites are components of the substrate binding site of SNase. These results indicate that these residues contribute to the binding both for FS and US, suggesting that the binding site topology is formed at the U state. The inhibitor in the US complex interacts with residues both in the N- and C-terminal sub-domains. We showed that the lack of W140 in SNase causes the loss of the essential non-local interaction, which leads to the loss of native structure. In the induced-folding process, the inhibitor binding compensates for the lost non local interaction to recover its foldability.

2P086 シトクロム c のモルテンングロビュール状態の熱力学的研究
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Thermodynamic study on the molten globule state of cytochrome c
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The molten globule (MG) states, structurally distinct from both the native (N) and denatured (D) states, of cytochrome c are stabilized under low pH and high anion concentration. Two different MG states (MG1, MG2) of cytochrome c were found at low pH and high salt condition. In this study, the stabilities of MG states of cytochrome c were evaluated under high and low salt condition. The N-MG1 transition of cytochrome c under high salt condition was directly observed by isothermal acid-titration calorimetry (IATC) and was well approximated to be a two-state transition. The thermal transition from MG1 to D state under high salt condition was evaluated by DSC, IATC and pressure perturbation calorimetry (PPC). It was clearly confirmed that the cooperative transition from MG1 to MG2, and from MG2 to D. Thermodynamic parameters between N, MG1, MG2, and D state were evaluated. It suggests that these MG states retain about 50% of native-like hydrophobic core. The stability of MG state under low salt condition was evaluated by calorimetry. The structure and energetic properties of this MG state was close to MG1 state observed under high salt condition. The phase diagram of cytochrome c under low salt condition showed that the MG state existed stably in higher pH than pH 4 at 70degreeC. The Gibbs free energy change in N-MG transition and that in N-D transition were evaluated. This suggests the energetic implication that the equilibrium MG state is identical to the transient intermediate on folding.

2P085 水溶性セレン試薬を用いたリボヌクレアーゼ A の酸化的リフォールディング経路における安定な 3S 中間体の観測
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Observation of stable 3S intermediates on the oxidative folding pathways of ribonuclease A by using a water-soluble selenium reagent
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In the oxidative refolding pathways of proteins that have several SS linkages, SS formation and SS rearrangement reactions occur simultaneously with formation of the stable structures. However, when a water-soluble selenium reagent, called DHSox, is used as an oxidizing reagent, the SS rearrangement reactions can be observed separately because the SS formation reactions proceed very rapidly. Thus, we succeeded to observe the stable 3S intermediates of ribonuclease A (des[65-72] and des[40-95]) at 25C and pH 8 by using DHSox. In this paper, we studied on the effects of pH and temperature on the oxidative folding pathways of ribonuclease A. The SS formation reactions normally end up within 1 minute after addition of three equivalents of DHSox to the solution containing a reduced form of ribonuclease A (R), and the SS rearrangement reactions proceed subsequently among the produced R, 1S, 2S, 3S, and 4S intermediates. At 25C and pH 8, formation of des[65-72] and des[40-95] intermediates and the native form (N) was observed after 5h. At pH 10, the two 3S intermediates and N were formed faster, while they were not formed at acid pH's. On the other hand, the des[65-72] intermediate was produced mainly at higher temperatures, while at lower temperatures two new intermediates, which would be the des[26-84] and des[58-110] intermediates, were clearly observed. The results suggested that the oxidative folding pathways of ribonuclease A should change depending on pH and temperature.

2P087 プロリン残基を含まないスタフィロコッカス・ヌクレアーゼ変異体のアンフォールディング転移の解析
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Analysis of Unfolding Transition of a Proline-free Variant of Staphylococcal Nuclease
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Staphylococcal nuclease (SNase) is a 149-residue protein that has been one of the best-characterized proteins in studies of protein folding. The proline-free variant of SNase has played an important role in investigating the folding mechanisms of SNase because of the simpler folding kinetics than those of the wild type protein. In the present study, all the six proline residues found in the wild type protein are replaced by Ala, Thr or Gly (P11A/P31A/P42A/P47T/P56A/P117G).

We investigated the stability of a proline-free variant of SNase at a level of secondary structures using the native state hydrogen exchange combined with NMR, followed by the assignments of the majority of backbone ¹H, ¹⁵N and ¹³C resonance using typical triple resonance NMR measurements. On the basis of the resonance assignments of the variant, native-state hydrogen/deuterium exchange in combination with NMR spectroscopy were carried out at 25 °C and pH* 5.1. The results indicate that the three of the β-strands within the β-barrel domain, strands II, III and V are more stable than the other secondary structure elements. Interestingly, the apparent stability of the protein from hydrogen exchange is 1.7 kcal/mol greater than the stability determined by equilibrium unfolding studies followed by circular dichroism and fluorescence. The difference of stability between the native-state hydrogen/deuterium exchange study and the equilibrium studies will be discussed.