2P064 1H-3-Hydroxy-4-oxoquinaldine 2,4-Dioxygenase の構造安定性
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Stability and unfolding of 1H-3-Hydroxy-4-oxoquinaldine 2,4-Dioxygenase
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Stability, unfolding mechanism, spectroscopic, and structural characteristics of the oxidatively stable C69S variant (HodC) of 1H3-hydroxy-4-oxoquinaldine 2,4-dioxygenase (Hod) have been determined by circular dichroism (CD) spectroscopy at various conditions. At 298.15 K, the urea-induced denaturation of HodC could be fitted by 2 states model (N-U) and the obtained midpoint concentration (C_m) was about 2.9 M. However, the GdnHCl-induced denaturation had an apparent intermediate state and could be fitted by 3 state model (N-I-U). The obtained C_m was about 0.7 M (N-I) and 2.5 M (I-U), respectively. The thermal stability was significantly dependent of pH. The midpoint temperature (T_m) was maximized at around pH 8.0 and the HodC was denatured at pH 4. The estimated number of protons bound to and released from the native state on decreasing the pH 6.75 and increasing the pH 10.0 was estimated to about 1.6 and 0.5, respectively. In high concentration of sodium phosphate buffer, the T_m of HodC was significantly increased. The increase indicates that the phosphate molecule bound relatively to the folding state of HodC. These results will be compared with fluorescence, sound velocity, and DSC results.

2P066 150℃ 近くに変性温度をもつ超好熱菌由来 CutA1 の熱安定性に果たす表面電荷残基の役割
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Role of surface charged residues in the thermostability of CutA1 from a hyperthermophile, with a denaturation temperature of nearly 150°C.
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We have found that CutA1 from *P. horikoshii* (PhCutA1) shows unusually high stability at neutral pH with a denaturation temperature of nearly 150°C which exceeds the highest record determined by DSC by about 30°C. From the comparison of crystal structures of three CutA1s from different sources with different growth temperatures, it has been revealed that extremely high stability of PhCutA1 has been caused by the remarkably increased number of ion pairs within the monomer structure. Therefore, in order to confirm the role of surface charged residues in the extremely high stability, we constructed mutant proteins of PhCutA1 at Glu or Asp residues that interact with counter charged residues (Lys or Arg) less than 4 Å on the surface. We examined stability changes of 22 single substitution mutants (D to N/A, or E to Q/A) at 11 positions by DSC. Under the neutral conditions, 15 mutants showed the significant reduction of the denaturation temperatures (between 0.5 and 8.9°C). At the acidic region, 8 mutants showed the decrease in stability, while 12 mutants increased the stability, suggesting that charged residues do not contribute to the stability due to protonation at pH 2.5. These results have confirmed our stabilization mechanism from the analyses of X-ray structures. We will furthermore confirm it using multiple mutants from above single mutations and other mutations at charged residues.

2P065 高温細菌 *Hydrogenophilus thermoluteolus* 由来シトクロム *c* の安定化機構
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Molecular Dissection for the Stability Mechanism of Cytochrome *c* from a Moderately Thermophilic Bacterium, *Hydrogenophilus thermoluteolus*
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Cytochrome c_{552} (PH c_{552}) from a moderately thermophilic *Hydrogenophilus thermoluteolus* has intermediate stability between homologous cytochrome c_{552} (HT c_{552}) of thermophilic *Hydrogenobacter thermophilus* and cytochrome c_{551} (PA c_{551}) from mesophilic *Pseudomonas aeruginosa*. To understand the stability mechanism in PH c_{552} , we systematically mutated five amino acid residues, which have been predicted to affect the overall stability [Nakamura, S. *et al.* (2006) *Biochemistry* **45**, 6115-6123]. When PH c_{552} Lys-43 and Val-78 were mutated to Tyr and Ile, respectively, which occupied at the corresponding positions of the more stable HT c_{552} , the PH c_{552} variants increased its stability during guanidine hydrochloride denaturation to the same extent as that of HT c_{552} in terms of Gibbs free energy change. In contrast, mutations of Ala-7, Met-13, and Tyr-34 in PH c_{552} to the corresponding residues in the less stable PA c_{551} (Phe, Val, and Phe, respectively) resulted in the destabilization. These results suggest that the intermediate stability of PH c_{552} is attributed to the selection of amino acid residues at these five positions. In addition, we found that Lys-43 contributed to the stability through forming an attractive electrostatic interaction with Asp-40, which did not occur in the corresponding region of HT c_{552} . Therefore, PH c_{552} has a unique strategy for increasing the stability that had not been adopted by the naturally occurring and highly stable HT c_{552} .

2P067 翻訳開始因子サブユニットの会合の熱力学的解析
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Thermodynamic analysis of the assembly of the α -, β -, γ -subunits of archaeal initiation factor 2
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In the initiation of translation, archaeal initiation factor 2 (aIF2), which is a heterotrimeric protein consisting of α -, β -, γ -subunits, delivers an initiator methionine tRNA (Met-tRNAi) to a small ribosomal subunit and positions them on the initiation codon of an mRNA. It is known that, in delivering step, aIF2 makes a ternary complex with Met-tRNAi and GTP, and after the correct pairing between the AUG initiation codon on mRNA and the CAU anticodon of the Met-tRNAi has been assessed, GTP is hydrolyzed and aIF2:GDP is released from the ribosome. However, the detailed mechanism of the trimerization of the subunits and complexation with GTP remains to be clarified. In this study, we examined the thermodynamic parameter of the assembly of the subunits from *Sulfolobus (S.) solfataricus* with isothermal titration calorimetry (ITC) and the stability of the subunits with differential scanning calorimetry (DSC). In ITC measurement, the enthalpy change of the interaction between α - and γ -subunits in the presence of β -subunit was identical to that without β -subunit, suggesting that α - or β -subunits make complex with γ -subunit independently. Furthermore, in DSC measurement, γ -subunit denatured at less than 70 °C despite *S. solfataricus* grows under about 80 °C and the complex of the subunits denatured higher degree than it. These results may suggest that the subunits of aIF2 assemble immediately after production in vivo. In the presentation, we will also report effects of the presence of GTP in the trimerization and stability of the subunits.