論文 / 著書情報
Article / Book Information

題目(和文)	出芽酵母の脱凝集シャペロンHsp104と基質タンパク質の相互作用機序の解明
Title(English)	
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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Misfolded or aggregated protein impairs cellular protein homeostasis, proteostasis. To prevent the accumulation of aggregated proteins, molecular chaperones promote protein folding in the cell. Moreover, some chaperones have an ability to solubilize aggregated proteins. AAA+ chaperone Hsp104 in budding yeast can solubilize and reactivate protein aggregates in cooperation with Hsp70/40 (Ssa1/Ydj1). Hsp104 is also required for the stable propagation of yeast prions by remodeling amyloid aggregates. Hsp104 forms hexameric ring structures and translocates substrate polypeptides through its central pore using energy from ATP hydrolysis. So far, the disaggregation mechanism of Hsp104 has been revealed by biochemical assays, which mainly rely on an appearance of substrate activity after the disaggregation reaction by the chaperones. However, the process by which Hsp104/Ssa1/Ydj1 solubilize the substrate aggregates remains poorly understood. Alternatively, the interaction between Hsp104 and substrates has been analyzed using model substrates or *in vivo* imaging. However, the detailed mechanism of interaction between Hsp104 and the substrate or the dynamics of Hsp104 are not revealed. In this research, I focused on the process of the disaggregase reaction by Hsp104/Ssa1/Ydj1. I analyzed the tendency of Hsp104 to recognize amino acids using peptides that confer prion properties on polyglutamine. Moreover, I developed the experimental system to analyze the disaggregase reaction at single-molecule level to characterize the size distribution of aggregates and to understand the dynamics of Hsp104 interacted with aggregates.

In chapter 1, the overview of protein folding and molecular chaperone was reviewed. The function and structure of Hsp104 were described. The purpose and significance of this research were described.

In chapter 2, to elucidate how Hsp104 recognizes the substrate, the interaction between Hsp104 and the peptides that converted the nontransmissible polyglutamine to transmissible aggregates were analyzed. I purified GFP fused with the peptides or mutants in which hydrophobic and aromatic amino acids were replaced with Ala, Ser and Thr. When the interaction between Hsp104 and GFP fused with the peptides was analyzed using size exclusion chromatography, we detected the binding of peptides and mutants to Hsp104. To evaluate the interaction quantitatively, I used fluorescence correlation spectroscopy (FCS) and calculated dissociation constants (K_d). FCS analysis revealed that the affinity of the GFP fused with the peptides for Hsp104 was higher than that of mutant peptides. The result showed Hsp104 had a tendency to recognize hydrophobic amino acids.

In chapter 3, I developed the methods to reveal the size distribution of aggregates in disaggregase reaction. As previously reported, aggregated luciferase was reactivated in the presence of Hsp104/Ssa1/Ydj1. First, I estimated the size distribution of luciferase aggregates at single-particle level using highly inclined and laminated optical sheet illumination microscopy (HILO). The presence of Hsp104 did not change the size distribution of the aggregates. In contrast, the presence of Ssa1/Ydj1 or Hsp104/Ssa1/Ydj1 affected the size distribution of aggregates and reduced the number of large aggregates. However, the difference of size distribution between the presence of Ssa1/Ydj1 and Hsp104/Ssa1/Ydj1 was not detected. Second, I measured the luciferase aggregates using FCS to characterize the aggregates which were not detected using HILO and distinguished the size distribution of aggregates between Ssa1/Ydj1 and Hsp104/Ssa1/Ydj1. When the luciferase aggregates in the presence of Ssa1/Ydj1 were measured using FCS, the size of luciferase aggregates were not changed over time. In contrast, luciferase aggregates were small in the presence of Hsp104/Ssa1/Ydj1. In summary, I developed the methods to characterize the size distribution of luciferase aggregates.

In chapter 4, to reveal the dynamics of Hsp104 in the disaggregase reaction, I visualized the interaction between Hsp104 and aggregates at single-molecule level. First, I developed the experimental system to visualize association/dissociation events of Hsp104 on the luciferase aggregates using total internal reflection fluorescence microscopy (TIRFM). Cy5-labeled luciferase was immobilized on a glass surface through a biotin-neutravidin linker. When Cy3-labeled Hsp104 was added to Cy5-luciferase immobilized on the glass surface, I monitored Cy3-Hsp104 bound to and released from Cy5-luciferase aggregates. Second, I analyzed the frequency of binding/release events between Hsp104 and luciferase aggregates and association time (dwell time). The frequency of binding/release events was increased depends on the presence of Ssa1/Ydj1. The result of statistical analysis of dwell time showed the complex between Hsp104 and the aggregates decayed exponentially with time, irrespective of the presence of Ssa1/Ydj1. Moreover, this binding/release reaction was fit by two-components reaction; fast components (3~4 sec) and slow components (~30 sec). The fast and slow components of dwell times were not changed irrespective of the presence of Ssa1/Ydj1. However, the proportion of slow components was increased depends on the presence of Ssa1/Ydj1. Comparison between dwell times and ATP hydrolysis rate of Hsp104 suggested Hsp104 "processively" bind to aggregates, where Hsp104 associates with the aggregates without release in every single turnover.

In chapter 5, the conclusion remarks of this study and future perspectives were discussed.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
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