1P038

シヤペロニン GroEL の第二の ATP 結合部位の同定

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¹岡崎総合バイオ・戦略的方法論研究領域

Identification of the second ATP binding site of GroEL

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The chaperonin GroEL from *Escherichia coli* is a tetradecameric protein of 14 identical 57 kDa subunits arranged in two heptameric rings stacked back-to-back with a central cavity. The ATP-dependent control of the chaperone functions of GroEL is determined by two levels of allostery; one within each ring and the other between the rings, respectively. To simplify the complexity of this system, we have used a single ring variant of GroEL (SR1). We have investigated the ATP-induced allosteric transitions of single-tryptophan variants of SR1 by stopped-flow fluorescence measurement in the presence or absence of ADP, a well-known inhibitor of ATP. In the absence of ADP, the ATP concentration dependence of the rate constant of allosteric transition exhibits a multiple sigmoidal behavior. In the presence of excessive amount of ADP, on the other hand, the ATP concentration dependence of the rate constant shows a simple sigmoidal behavior. We also observed that with an increase in ADP concentration, the rate constant of the allosteric transition decreases in a multiple sigmoidal manner. These observations may be explained by assuming a second ATP binding site which is distinct from the known ATP binding site. We are in the process of identifying the second ATP binding site by labeling Y485W-SR1W with 8-azido-ADP.

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グループ II 型シヤペロニンのサブユニット間協同性における ATPase の役割

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Role of ATP hydrolysis in the cooperativity of subunits of group II chaperonins

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Group II chaperonins, found in the archaeal and eukaryotic cytosol, function independently of the cofactor corresponding to GroES of group I chaperonins. Rather, the helical protrusion at the tip of the apical domain, which forms a built-in lid of the central cavity, substitutes for the cofactor. We have been studying the protein folding mechanism of group II chaperonins using the chaperonin from a hyperthermophilic archaeum, *Thermococcus* sp. strain KS-1. Previous studies have shown that ATP drives the conformational change of group II chaperonins, from the open-lid, substrate binding conformation, to the close-lid conformation to encapsulate unfolded protein in the central cavity. However, the details of the ATP-driven reaction remain obscure. It has been considered that the conformational change is driven in the cooperative manner. However, the mechanism of the cooperation between subunits has not been studied. To elucidate this issue, we constructed and characterized the chaperonin complexes which are composed of wild and ATP hydrolysis-mutated subunits in the ordered fashion by connecting them. Consequently, the complexes containing mutations around ATP binding sites retained protein folding activity, and ATP dependent conformational change of these complexes occurred by small angle X-ray scattering (SAXS). Thus, we concluded that cooperative action of ATP hydrolysis in the ring is not important in the protein folding cycle of group II chaperonins.

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蛍光プローブを用いた Group II 型シヤペロニンの helical protrusion の動的解析

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Dynamic analysis of helical protrusion of group II chaperonins using fluorescent probes

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Group II chaperonins, found in the archaeal and eukaryotic cytosol, function independently of the cofactor corresponding to GroES of group I chaperonins. The helical protrusion at the tip of the apical domain, which forms a built-in lid of the central cavity, substitutes for the cofactor. We have been studying the protein folding mechanism of group II chaperonins using the chaperonin from a hyperthermophilic archaeum, *Thermococcus* sp. strain KS-1. In group II chaperonins, ATP drives the conformational change of group II chaperonins, from the open-lid, substrate binding conformation, to the closed-lid conformation to encapsulate unfolded protein in the central cavity. It has been considered that the dynamic changes of helical protrusion of each subunit contribute largely to ATP-dependent conformational change of oligomeric chaperonin, and furthermore, the conformational change is driven in the cooperative manner. However, the detailed mechanism of the cooperation between subunits has not been studied. To elucidate this issue, the chaperonin complexes were composed of wild-type, helical protrusion-deletion, and mutant with cysteine at the tip of the helical protrusion in the connected manner, and then they were subjected to protease digestion and labeling with fluorescent probe via cysteine. We examined the effect of ATP on locally conformational changes of helical protrusion of group II chaperonins using the fluorescent probes.

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如何にしてプロテアソームは分解する蛋白質を選ぶのか？

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1aF04

SELECTING PROTEINS FOR DEGRADATION: THE INITIATION STEP

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The ubiquitin-proteasome system is responsible for degradation of most proteins inside eukaryotic cells. Proteins are targeted to the proteasome by covalently attached polyubiquitin chains. However, effective degradation requires the presence of a second signal, an unstructured initiation site. We have characterized the requirement on the unstructured initiation site.

Ubiquitin modification and unstructured initiation site can be separated onto different polypeptide chains, but they have to be close to each other in space. We defined the constraints on this relationship by increasing distance between the ubiquitination and initiation sites in model substrates. Insertion of increasing numbers of titin domains showed that a separation of three or more domains prevented degradation completely. This inhibition can be overcome if the length of the initiation site is increased. This result indicates that the initiation site must be close to the ubiquitin modification in space to function.

We recently found that not all sequences can function as initiation sites. Comparison of the degradation rate of substrates with 15 different initiation sites found a correlation between the degradation rate and sequence complexity but not any other physical properties. We are now investigating a physical basis of this observation and propose that the proteasome cannot bind to simple sequences.

Therefore, these results suggest that the location and the sequence complexity of the unstructured initiation site may ultimately determine the protein's fate.