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論文の要旨
Summary (Abstract) of doctoral thesis contents

The naturally evolved protein biomolecules are highly sophisticated in structures with diverse properties. Majority of these biomolecules function in integrative systems rather than acting by itself. Most often these elements can interact with the external environment to form supramolecular complex architectures. The biomolecular assemblies are highly dynamic in nature and essentially contribute to regulation of diverse array of integrated cellular functions. To interpret the biological significance of those molecular assemblies in living systems, it is important to characterize their structural architectures and dynamics in detail.

In general, it is assumed that high sequence identity would give rise to similar structure and function. Moreover, many examples have been described of homologous proteins sharing common, distinct fold and function with sequence identity less than 20%. However, there are also examples of proteins with more than 50% sequence similarity having different folds and functions, indicating that the sequence similarity is not enough to predict functions of proteins. Therefore, determination of structures and dynamics of proteins is really necessary not only to understand their physiological functions but also to artificially optimize their structural mechanisms for designated functions.

In such context, I have chosen an archaeal homolog of proteasome-assembly chaperon as model to structural study. Accumulated evidence has recently revealed that formation of the eukaryotic 20S proteasome involving heteroheptameric α-ring organization is not a spontaneous process but requires at least five proteins operating as assembly chaperones. The assembly chaperone proteins Pba1 and Pba2 form a heterodimer and thereby provide a scaffold for the α-ring formation during the eukaryotic 20S proteasome organization. In contrast to the eukaryotic proteasomes, the archaeal 20S proteasome consists of much less divergent subunits, which spontaneously assemble without any assistance from the chaperones. However, recent bioinformatic analysis has identified PbaA and PbaB as Pba1-Pba2 homologs in archaea. It is therefore enigmatic how these archaeal homologs are involved in proteasome assembly, which presumably proceeds in an autonomous fashion in archaeal cells. To solve this paradox, detailed structural characterization about these protein structures is necessary because the simple structural homology thus cannot estimate and explain their functions. That is why I was highly motivated to provide the structural insights into the archaeal homologs of proteasome-assembly chaperone in my PhD thesis. Such structural revelation could also offer a key clue about how the structural features of molecular assembly chaperones are shared between archaea and eukaryotes from a viewpoint of the molecular evolution.

A recent study in our group has revealed that the extremophilic hyperthermophile archaeal species *Pyrococcus furiosus* PbaB forms a homotetrameric structure with elongated C-terminal segments and acts as an ATP-independent proteasome activator. In such framework, I attempted to characterize the structural features of PbaA from *Pyrococcus furiosus* by an integrative structural analysis using X-ray crystallography, high-speed atomic force field microscopy (HS-AFM), native mass spectrometry, electron microscopy (EM), and solution scattering.

My X-ray crystallographic data revealed that PbaA forms a homopentameric structure and its C-terminal segments harboring a proteasome activating motif are packed inside the core, implying that it has no binding capability to the 20S proteasome. Furthermore, I found that PbaA could form a homodecameric cage-like structure in another crystal form, indicating that the C-terminal segment of the PbaA protomer could be elongated. Indeed, solution scattering and HS-AFM data revealed that the PbaA homopentamer not only remain in a closed state but also exhibited an open conformation regarding its C-terminal segments. Inspection of all these results revealed that the archaeal homologs of assembly chaperones PbaA and PbaB are different from the eukaryotic counterparts in terms of their oligomeric states and biological functions, although their protomer structures are quite similar as expected from their amino acid sequence identity: While the eukaryotic proteasome assembly chaperones form heterodimers, the archaeal homologs PbaA and PbaB form homopentamer and homotetramer, respectively, even though the C-terminal proteasome-activating motifs are shared among these proteins. Furthermore, despite their similarity in domain conformation, PbaA and PbaB are likely to exert distinct functions. Apparently from the crystallographic data, the PbaA homopentamer cannot bind the 20S proteasome as its C-terminal segments are primarily packed inside whereas the PbaB homotetramer can activate the 20S proteasome through its extended C-terminal segments. Unlike PbaB, PbaA can exhibit conformational transition between major close and minor open states regarding its C-terminal segments.

Based on the structural information of PbaA which I revealed, a protein engineering approach was applied with attempt to endow this protein with the binding ability to the 20S proteasome. Notwithstanding the fact that the C-terminal proteasome-activating motif is conserved between PbaA and PbaB, only PbaB can activate the 20S proteasome. By grafting the C-terminal 43 amino acid residues of PbaB to its C-terminal part, PbaA became capable of activating the 20S proteasome. This result confirmed that not only proteasome activating motif but also an open conformational state of the C-terminal segments is necessary to act as a proteasome activator. This approach will be applied to design and create novel protein functions on the basis of the homooligomeric scaffold of PbaA and PbaB.

Moreover, distinct structural architecture of PbaA suggests its intriguing structural mechanism associated with an as yet undiscovered function. In fact, a structural genomics report identified a putative binding protein PF0014 which makes complex with PbaA. Thus, I attempted to perform structural characterization of the complex formed between PbaA and PF0014. The native mass spectrometric study indicated that PbaA and PF0014 can make a complex in a 10:10 stoichiometry. The solution scattering and AFM data showed that this complex had a dumbbell-shaped structure. The detailed three-dimensional structure of PbaA-PF0014 complex was determined by EM analysis, indicating that its molecular construction resembles a classical Greek "tholos" where the PF0014 proteins bound to the PbaA homopentamers mimic the columns of this unique architecture through intermolecular interactions. These multiple structural architectures of PbaA and the PbaA-PF0014 complex suggest their unknown functions in archaeal cells, independent of the assembly and activation of the 20S proteasome. Thus, PbaA mainly forms homopentemer in solution and can form homodecamer in the crystal and the tholos-like complex with its binding partner. The various assembly states of PbaA can provide a new direction to think why this complexity does exist or whether it has some sophisticated novel functional roles in the living system. For example, because of its conformational versatility, PbaA may form different oligomeric structures in response to changes in environment surrounding the organism.

In summary, this study revealed unique, multiple structural architectures involving the archaeal homologs of proteasome assembly chaperones, giving new insights into the structural design underlying the dynamic ordering of biomolecules that have internal complexities for the creation of integrated functions.

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博士論文審査結果の要旨
Summary of the results of the doctoral thesis screening

生命体を構成しているタンパク質は、ダイナミックな構造変化を通じて固有の集合体形成を実現し、これにより高次の機能を発現していることが多い。こうしたタンパク質機能の発現メカニズムを明らかにするためには、タンパク質の高次構造を多角的に解析することが不可欠である。本論文は、プロテアソーム集合シャペロンの古細菌ホモログに着目して、その分子機能の理解を目的として構造研究を行ったものである。

第1章では、研究の背景と目的について以下の趣旨が述べられている。真核生物では細胞内のタンパク質分解装置であるプロテアソームの構造形成に集合シャペロンの介助を必要とする。一方、古細菌のプロテアソームは集合シャペロンの介助なしに自律的に形成される。それにもかかわらず、古細菌にはPbaAとPbaBとよばれる2種類の集合シャペロンのホモログが存在しており、これらのタンパク質はいずれもC末端部にプロテアソームと結合するモチーフを有している。PbaBはホモ4量体を形成してプロテアソームを活性化する機能を持つことが先行研究で示されたが、PbaAはプロテアソームに対する結合能を持たず、その構造機能は依然不明のままである。こうした状況に鑑みて出願者は、高次構造解析を通じてこの問題に取り組むことを構想した。

第2章では、X線結晶構造解析によってPbaAの3次元構造を明らかにした。PbaA は、PbaBとは異なりホモ5量体構造を形成していたが、各サブユニットのC末端側に位置するαヘリックスの配向は2つの結晶形によって異なっていた。すなわち、C末端ヘリックスが5量体のコア部分の疎水性表面を覆うように折りたたまれた閉構造と、それらがコアから突き出した開構造の2通りの構造が見出されたが、溶液散乱と原子間力顕微鏡（AFM）を用いた観測により、溶液中ではPbaAは主に閉構造を形成していることを示した。これにより、PbaAがプロテアソームに結合能を示さない理由が明らかとなった。

続く第3章では、構造情報に基づいた分子の設計・改変を通じてPbaAにプロテアソーム結合能を賦与することに成功している。すなわち、PbaAのC末端ヘリックスをPbaBの対応する部分と入れ替えることにより作製したキメラタンパク質では開構造の割合が増大しており、プロテアソームに対する結合能を獲得していることが溶液散乱と高速AFM観測により明らかとなった。実際に、生化学実験により、作出したキメラタンパク質がプロテアソーム活性化能を有していることも示された。

第4章では、古細菌中にPbaAと相互作用し得るタンパク質PF0014が存在することに着目し、両タンパク質の複合体の構造解析を実施している。ネイティブ質量分析とAFMおよび電子顕微鏡解析の結果、PbaAとPF0014は10分子:10分子からなるダンベル状の巨大な構造体を形成していることが明らかとなった。この構造体形成には、PbaAのC末端ヘリックスの構造変化を伴うことも示唆された。

最終章では、研究成果を総括するとともに、今後の展望が述べられている。本論文の研究成果により、共通の機能モチーフと相同な基本構造を有するタンパク質サブユニットが、立体構造の多型性を示し得る実例が示され、それが異なる機能発現の基盤となっていることが明らかとなった。また、高次構造解析の結果に基づいたタンパク質の機能改変にも成

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功している。

以上のように、出願者は主体的に研究に取り組み、それによりタンパク質機能の分子科学的理解を深める知見を与えている。また、本論文の第 2 章の一部は既に国際学術雑誌に報告されており、本審査委員会は本論文が博士（理学）の授与に値すると全員一致で判断した。