Multiple structural architectures of archaeal homolog of proteasome-assembly chaperone

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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 including X-ray crystallography.

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. 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.

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 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.