PD Research Report for the 2016 year

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Research Theme Development of Novel Apparatus For Protein Trapping Using Thermostable Chaperonin
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Research Results（about 2,500 characters in Japanese, about 65 lines times 90 characters in English）

Our work is about developing a novel apparatus for protein trapping through archaeal chaperonin.

Molecular chaperonin CpkB from Thermococcus kodakarensis possesses a unique negatively charged carboxy-terminal region that functions in target protein recognition. In the present study, green fluorescent protein (GFP), 4-oxalocrotonate tautomerase (4OTA) and glutamine:fructose-6-phosphate amidotransferase (GFAT) were fused with a positively charged tag, which was selected by docking simulation in silico, to enhance electrostatic interactions with CpkB. Target proteins were heated at 75°C in the presence or absence of CpkB, and the remaining enzymatic activity was measured. The half-life (*t1/2*) of tag-fused target proteins was significantly extended in the presence of CpkB comparing to tagless targets. Escherichia coli cell extracts containing heterologously expressed targets (GFP, 4OTA and GFAT and their tagged variants) were incubated at 75°C in the presence or absence of CpkB, and the proportion remaining in the soluble fraction was evaluated by SDS-PAGE. All tag-fused targets remained predominantly in the soluble fraction in the presence of CpkB, but were barely noticeable in the absence of CpkB, suggesting that CpkB protected tagged proteins more efficiently than non-tagged targets. Attachment of a positively charged tag may be a generally applicable method for enhancing recognition by CpkB.

Chaperones are generally considered to catalyze the folding of a wide range of target proteins due to low specificity. Group I chaperonins such as GroEL/ES and some group II chaperonins are highly promiscuous and target a broad range of substrates through hydrophobic interactions, but they exhibit a weak bias towards several structural fold types. By contrast, the group II ring complex/chaperonin TCP-1 (TRiC/CCT) displays higher specificity towards particular proteins through charged and polar interactions between substrates and apical domains of TRiC/CCT subunits. Group II chaperonin

thermosomes assemble into a similar TRiC/CCT structure, and their apical domains are also believed to function in substrate recognition. However, the apical domain of thermosomes is less well studied than TRiC/CCT.

The carboxy-terminal regions of chaperonins are highly variable in amino acid sequences and are believed to function in ATP hydrolysis and substrate binding. The hyperthermophilic archaeon Thermococcus kodakarensis grows optimally at 85°C and possesses two chaperonins, the cold-inducible CpkA and the heat-inducible CpkB, which are involved in adaptation to low and high temperatures, respectively. These two chaperonins share high sequence identity (77%), but differ significantly in their carboxy-terminal regions. CpkA differs from CpkB by possessing a mildly hydrophobic Gly-Gly-Met (GGM) repeat sequence in its carboxy-terminal region, similar to that originally identified in Escherichia coli GroEL. By contrast, CpkB has a classic thermosome carboxy-terminal region that is rich in negatively charged residues. In this study, we attempted to enhance the affinity of CpkB for its target proteins by introducing a positively charged tag through electrostatic interaction between this positive charged tag and negative charged C-terminal region of CpkB.

In summary, we showed that the specificity of a group II chaperonin was improved by increasing electrostatic interactions with the target protein by attaching a positively charged tag to the target protein that can interact with the negatively charged carboxy-terminal region of CpkB. Electrostatic interactions could potentially be further enhanced by introducing additional negatively charged residues into the recognition domain of CpkB.

In this year, we have submitted one paper about this theme to the journal of Bioscience and Bioengineering，one Springer book chapter about C-terminal region of archaeal chaperonins and their classification, and one biotechnology patent for signal tag – chaperonin system.