PD Research Report for the 2014 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）

To develop a novel apparatus, we improved the CpkA's activity this year. *Thermococcus kodakarensis* optimally grows at 85˚C and possesses two chaperonins, cold-inducible CpkA and heat-inducible CpkB, which are involved in low-temperature and high-temperature adaptations, respectively. These two share a high sequence identity (77%) except their C-terminal regions. CpkA contained "GGM" motif shows the highest ATPase activity at 60˚C in contrast to CpkB which shows the highest activity at higher than 90˚C. To clarify the effect of shifted ATPase activity to lower temperature on chaperonin function, various CpkA mutants were constructed by introducing mutations into its C-terminal region. CpkA mutant whose original Glu530 was replaced with Gly (CpkA-E530G) showed the increased level of ATPase activity and the highest activity was detected at 50˚C. The efficacies of CpkA mutants on denatured indole-3-glycerol-phosphate synthase ($TrpC_{Tk}$), which is known as a CpkA target, were examined in vitro. CpkA-E530G assisted chemically unfolded $TrpC_{Tk}$ to refold at 50˚C more efficiently than CpkA. Effect of cpkA-E530G mutation on cell growth was then investigated by introducing cpkA-E530G into the genome of *T. kodakarensis* KU216 (*pyrF*). The mutant strain DA4 (*pyrF*, *cpkA*-E530G) grew as well as parental strain KU216 at 60˚C. By contrast, DA4 grew more efficiently than KU216 at 50˚C. The obtained results suggested that mutation CpkA-E530G prevented cold denaturation of proteins at cold-stressed condition and enabled cells to grow at cooler environments. It is noteworthy that only one base pair substitution in chaperonin offers cell growth dominancy at a new environment.

In the present study, we constructed eight mutants harboring replacements of four amino acid residues in the C-terminal region of CpkA to enrich "GGM" motif. Purified CpkA-E530G showed an enhanced ATPase activity and chaperonin activity at 50˚C in vitro. Cold adaptation of *T. kodakarensis* is a multi-loci controlled phenomenon which is adjusted through many mechanisms including cell membrane constitution, DEAD-box RNA helicase, chaperonins, and transcription factors. It is believed that the beneficial mutations are accumulated incrementally in each mechanism and synergistically adapt cold environment. However, when we introduced cpkA-E530G into the genome of *T. kodakarensis*, to our surprise, by just one single base pair substitution, this mutation conferred *T. kodakarensis* more adaptability at 50˚C which grew much faster than parent strain KU216. Enhanced cold adaptability is believed to be correlated with improved ATPase activity of chaperonin at 50˚C. C-terminal region of GroEL is related to the ATPase activity and substrate binding. It is reported that CpkA showed a similar bias of substrate preference with GroEL. In this study, we showed that ATPase activity and chaperonin activity of CpkA were also affected by its C-terminal region. According to our results shown here, the

location of amino acids in C-terminal region is the primary factor to affect chaperonin function: Glu530, which is in a moderate distance (~20 Å) to the ATPase functional core area of CpkA, played a positive role in the mutations when it is replaced by either Gly or Met. The second factor is the type of residue at C-terminal region. Gly was more favored at 50˚C than Met to enhance ATPase activity. Generally Gly, Pro, Ser, and Ala are most represented amino acids while Met, Ile, and Trp are largely absent. All three Gly replacements of polar residues (E530G, Q538G, and D545G) increased the ATPase activities at 50˚C, while the Met replacements maintained the activities above 60˚C. Functional movements of catalytic core of ATPase activity is involved in catalytic rate and substrate binding in chaperonin α-subunit of *Thermococcus* KS-1. Glu530 substitution with Gly in CpkA would remove any possible blemish so that the activity was further promoted. Pro residue is usually considered as a "turn maker or helix breaker" to provide local structure of a protein with extra rigidity due to its secondary amine structure with reduced freedoms (i.e. smaller free entropy). Replacement of Pro538 with either Gly or Met (CpkA-P538G or CpkA-P538M) would change the trend of peptide backbone and lead C-tail to an incorrect direction, which would decrease ATPase activity.

In summary, two trends existed in group II chaperonins along the growth temperature were revealed. This divergence has been driven by the duplication of an additional chaperonin gene firstly and by mutation of this gene subsequently. Tandem repeats is believed as a mutational hotspot to evolve. C-terminal region of CpkA of which DNA sequence is coding tamdemly repeated (GGM motif). We showed here that *T. kodakarensis* could adapt colder growth temperature (50˚C) by only one base pair substitution in C-terminal region of CpkA (CpkA-E530G). Due to that chaperon play as a network integrator of protein-protein interaction (PPI) network in the cells, especially under the stresses, any change of it would affect its downstream proteins of PPI network and subsequently lead changes of cell phenomena, such as cold adaptation. Moreover GroEL is reported to pronouncedly affect the refolding of proteins called mutators which are involved in DNA repair so that GroEL would accelerate the mutation rate all over the host cell. Considering the similar substrates bias between GroEL and CpkA, CpkA is expected to play a resemble role of GroEL in *T. kodakarensis*. Further *in vivo* studies are worthy to carry out to obtain a mesophilic lineage from hyperthermophiles to better understand adaptive mechanism applied in archaea.

List of research achievements

1. Hidese, R., R. Nishikawa, Le Gao, M. Katano, T. Imai, S. Kato, T. Kanai, H. Atomi, T. Imanaka, and S. Fujiwara. Different roles of two transcription factor B proteins in the hyperthermophilic archaeon *Thermococcus kodakarensis*. Extremophiles 18:573-88. 2014.
2. A single amino acid substitution of thermosome assigned a cold adaptation in hyperthermophile. The 66nd Annual Meeting of Society for Biotechnology of Japan. Sapporo, September 9-11, 2014. (Poster)
3. Mutant chaperonin functional at lower temperature enables cells grow at further cold-stressed temperature in hyperthermophilic archaea. Le Gao, Tadayuki Imanaka and Shinsuke Fujiwara (in submission)