Adaptation of a hyperthermophilic group II chaperonins to relatively moderate temperatures

Chaperonins constitute one of the principal molecular chaperone families. They are ubiquitous and take indispensable roles in protein folding in cells. They form large cylindrical complexes composed of two stacked rings of 7-9 subunits of about 60 kDa. Each ring has a large central cavity for the productive folding of a non-native protein in an ATP-dependent manner. On the basis of their protein sequence and structural features, chaperonins are divided into two groups: I and II [1]. Group I chaperonins including GroEL of *Escherichia coli* are found in bacteria and endosymbiotic organelles. On the other hand, group II chaperonins are found in archaea and the eukaryotic cytosol. The most important difference between them is the requirement of a co-chaperonin, GroES. GroES acts as a detachable lid for group I chaperonins by forming a heptameric ring-shaped structure. In contrast, group II chaperonins have a built-in lid called the helical protrusion, which is composed of an extension of the apical domain (Fig. 1) [2]. The helical protrusion plays the equivalent role of GroES, sealing off the central cavity of the chaperonin complex. We have been studying the reaction mechanism of group II chaperonins using that from a hyperthermophilic archaeon, *Thermococcus* sp. strain KS-1 (*T.* KS-1) [3]. Although *T.* KS-1 chaperonin provided advantages for our study in terms of its high stability and activity, its high thermophilicity caused difficulty in using various analytical methods. To resolve this problem, we tried to adapt *T.* KS-1 chaperonin to moderate temperatures by mutations [4]. A comparison of the amino acid sequences between 26 thermophilic and 17 mesophilic chaperonins showed that three amino acid replacements (187th, 323rd, and 523rd residues of the *T.* KS-1 α subunit) are likely to be responsible for the difference between their optimal temperatures. Among thermophilic chaperonins, E187, K323, and A523 were strongly conserved. On the other hand, among mesophilic chaperonins, the residues are substituted for D, R, and K. The locations of these amino acid residues are displayed in the closed conformation of *T.* KS-1 chaperonin (Fig. 1). E187 is located in the β-sheet of the intermediate domain and K323 in an α-helix in the bottom of the apical domain. These amino acids are located in the hinge region between the intermediate and apical domains. In contrast, A523 is located at a β-sheet of the resolvable C-terminal region in the crystal structure, which constitutes the bottom of the cavity. We introduced amino acid replacements in three amino acid residues of *T.* KS-1 chaperonin and examined whether they adapted to cold temperature.

To examine the folding activities of the mutants, protein folding assay was carried out at 60°C, 50°C, and 40°C using citrate synthase from *Thermoplasma acidophilum* (TCS) (Fig. 2). The refolding activity of all the single mutants at 60°C was lower than that of the wild type. On the other hand, only K323R exhibited improved folding activity at lower temperatures such as 50°C and 40°C. E187D did not exhibit significant improvement in folding activity at 50°C. The activities of A523K at 50°C and 40°C were lower than those of the wild type.

Group II chaperonins take the open conformation in the absence of ATP, and they change to the closed conformation after ATP binding and hydrolysis. The

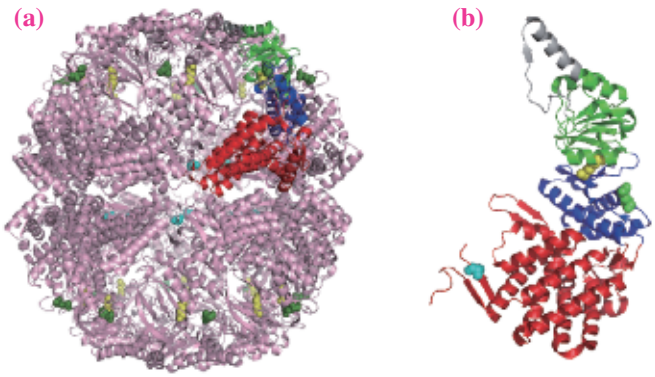

Fig. 1. Location of amino acids mutated for cold adaptation. Crystal structures of *T.* KS-1 α chaperonin oligomer **(a)** and α subunit **(b)** in the closed conformation. The equatorial, intermediate, and apical domains are shown in red, blue, and green, respectively. The helical protrusion, which is located at the tip of the apical domain, is shown in silver. The mutated residues for cold adaptation, i.e., E187, K323, and A523, are shown as Corey-Pauling-Koltun (CPK) style in green, yellow, and cyan, respectively. In **(a)**, only one subunit is shown in the above colors, and the mutated residues within a chaperonin oligomer are shown as CPK style in colors corresponding to E187, K323, and A523.

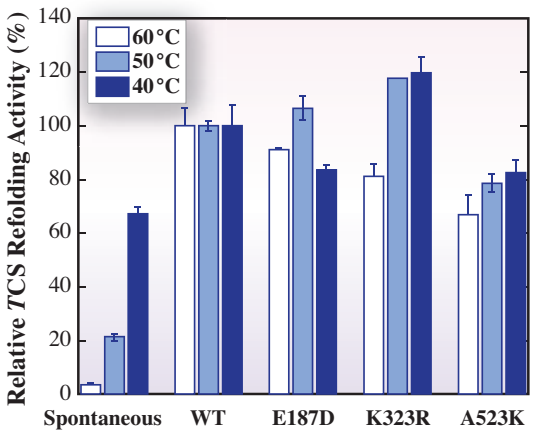

Fig. 2. *Thermoplasma* citrate synthase refolding activities of mutant chaperonins. Refolding activities for *Thermoplasma* citrate synthase (*T*SC) of chaperonin variants were determined at 60°C, 50°C, and 40°C. The activities of *T*CS refolded by wild-type chaperonin (WT) at 60°C, 50°C, and 40°C were taken as 100% (0.30, 0.27, 0.22 mU, respectively). Error bars indicate standard deviation (n = 5).

ATP-dependent conformational change ability of mutant chaperonins was examined by small angle X-ray scattering (SAXS) experiments at 60°C and 40°C (Table 1). All mutants exhibited ATP-dependent differences in the scattering curves at both temperatures. At 40°C, the ATP-dependent differences in the scattering curves of K323R were clearly larger than those of the wild type and other mutant chaperonins. SAXS gives the size of the molecule using two structural parameters, the radius of gyration (R_g) and the maximum particle distance (D_{max}). R_g indicates the mean particle size, and D_{max} is the maximum particle distance or the maximal intramolecular distance. At 60°C, ATP-dependent changes in D_{max} and R_g (ΔR_g) were observed in all chaperonins. There was no significant difference in ΔR_g among the chaperonins. The ATP-dependent ΔR_g parameters for the wild type, E187D, K323R, and A523K were -7.4 Å, -6.7 Å, -6.5 Å, and -7.4 Å, respectively. At 40°C, the ATP-dependent ΔR_g parameters for the wild type, E187D, K323R, and A523K were -3.8 Å, -5.3 Å, -6.6 Å, and -4.5 Å, respectively. These results showed that the ATP-dependent conformational change abilities of the wild type and A523K reduced at 40°C, correlating with their weak folding activities at that temperature. In contrast, K323R possessed the same ATP-dependent conformational change ability at 40°C and 60°C. The difference between the ΔR_g values of E187D at 60°C and 40°C seemed to be small. The mutation sites of E187D and K323R are located in the hinge region between the intermediate and apical domains. Considering that the conformational change is induced from the equatorial domain to the apical domain, SAXS experiments suggested that the amino acid substitutions in the hinge region, such as K323R, are important for the conformational change at relatively moderate temperatures. Since K323 may secure helix 12 in the closed conformation by interacting with D198, the replacement of Lys with Arg is likely to induce a higher mobility of the built-in lid, resulting in higher activities at relatively low temperatures.

Although the cold adaptation of the mutants was not sufficient for practical application, the results gave important information on the protein folding mechanism of group II chaperonins.

The experiments were performed at beamline **BL40B2**.

Table 1: Structural parameters of wild-type and single mutant chaperonins determined by small angle X-ray scattering experiments at 60ºC and 40ºC

Chaperonin	Nucleotide	60 °C			40 °C		
		R_g (Å)	ΔR_g (Å)	D_{max} (Å)	R_g (Å)	ΔR_g (Å)	D_{max} (Å)
Wild type	Free	75.4	—	191	76.6	—	198
	+ATP	68.1	−7.3	170	72.8	−3.8	192
	+ADP	76.0	0.6	196	75.2	−1.4	195
E187D	Free	74.6	—	195	74.5	—	194
	+ATP	67.9	−6.7	169	69.2	−5.3	182
	+ADP	73.9	−0.7	195	73.6	−0.9	196
K323R	Free	74.3	—	191	74.4	—	195
	+ATP	67.8	−6.5	170	67.8	−6.6	169
	+ADP	73.5	−0.8	189	74.7	0.3	195
A523K	Free	75.7	—	194	75.7	—	197
	+ATP	68.3	−7.4	171	71.2	−4.5	180
	+ADP	74.9	−0.8	193	75.4	−0.3	188

Error values of delta-radius of gyration (ΔR_g) and maximum particle distance (D_{max}) were estimated to be about ±1.0 Å, and ±5%, respectively.

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