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Crystallization and preliminary X-ray characterization of archaeal group II chaperonin α-subunit from *Thermococcus* strain KS-1

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The archaeal group II chaperonin from *Thermococcus* strain KS-1 is composed of two kinds of subunits (α and β). Each of the recombinant subunits was individually expressed in *Escherichia coli* and purified as homo-hexadecamers of each subunit. Both homo-oligomers facilitate the refolding of denatured proteins *in vitro* in an ATP-dependent manner. A mutant α-subunit homo-oligomer with two amino-acid substitutions, which has the ability to capture the unfolded protein but lacks the ability to refold the unfolded protein, was crystallized in two different conditions. One crystal form was obtained from a high-concentration solution of ammonium sulfate and grew to maximum dimensions of 0.15 × 0.15 × 0.4 mm. The crystals of this form belonged to the tetragonal space group $P42_12$, with unit-cell parameters $a = b = 209.3$, $c = 156.1$ Å, and diffracted X-rays to 2.4 Å resolution with synchrotron radiation. The other form was crystallized from a polyethylene glycol 6000 solution and belonged to the tetragonal space group, with unit-cell parameters $a = b = 220.8$, $c = 182.4$ Å. This form only diffracts X-rays to 6 Å resolution. Diffraction data collected from the former crystal enabled initial successful phases to be obtained by the molecular-replacement method.

1. Introduction

The molecular chaperones chaperonins assist the refolding of denatured proteins in an ATP-dependent manner and consist of double-ring assemblies, both of which are comprised of several subunits (Hemmingsen *et al.*, 1988). Based on their amino-acid sequences, chaperonins are classified into two groups (Gupta, 1995): group I chaperonins exist in eubacteria (Georgopoulos *et al.*, 1973), mitochondria (Cheng *et al.*, 1989) and chloroplasts (Hemmingsen *et al.*, 1988), whereas group II chaperonins are found in archaea (Phipps *et al.*, 1991) and the cytoplasm of eukaryotic cells (Frydman *et al.*, 1992; Gao *et al.*, 1992). There are several significant differences between chaperonins of groups I and II. Firstly, most group I chaperonins are composed of a single kind of subunit and form a heptameric double-ring structure (Fenton & Horwich, 1997; Hartl, 1996; Sigler *et al.*, 1998), while group II chaperonins consist of up to eight different kinds of subunits and form an octameric or nonameric double-ring (Archibald *et al.*, 1999; Gutsche *et al.*, 1999). Secondly, GroEL from *Escherichia coli*, which is the best characterized group I chaperonin, cooperates with the heptameric cofactor (GroES), whereas no such cofactor has been found in the group II chaperonins. X-ray crystallography has revealed that the chaperonin subunit is composed of apical, intermediate and equatorial domains (Braig *et al.*, 1994; Ditzel *et al.*, 1998). The crystallography of an archaeal chaperonin (thermosome) from *Thermoplasma acidophilum* (Ditzel *et al.*, 1998) and the apical domain of its α-subunit (Klumpp *et al.*, 1997) indicated that both α- and β-subunits have a protrusion segment in their apical domains that seems to correspond to GroES. In addition, the overall structure of the group II chaperonin is similar to that of the GroEL–ES complex in its spherical shape, rather than to that of apo-GroEL. This would imply that the group II chaperonin itself possesses a lid domain and functions without any cofactors. These differences suggest that the mechanism underlying protein folding by the group II chaperonins is considerably different from that of the group I chaperonins. However, compared with the group I chaperonins, which are well characterized both structurally and functionally, little is known regarding the biological and structural aspects of the group II chaperonins.

The hyperthermophilic archaea *Thermococcus* strain KS-1 (*T.* KS-1) produces a hexadecameric group II chaperonin composed of α-subunits (molecular weight 59.1 kDa; SWISS-PROT ID O24729) and β-subunits (molecular weight 59.2 kDa; SWISS-PROT ID

O24730). Both of the recombinant subunits are capable of forming homo-hexadecameric oligomers and have the ability to refold non-native proteins (Yoshida *et al.*, 1997, 2000, 2002). In spite of the high identity in amino-acid sequence between the two subunits (80.9%), there are some differences, for example in thermostability (Yoshida *et al.*, 2001, 2002). In addition to the wild-type α-subunit, we have cloned a mutant (G65C/I125T) which has no ATP-dependent refolding activity but has the ability to capture denatured proteins (Yoshida *et al.*, 1997, 2000, 2002; Iizuka *et al.*, 2001). Because of this unique character, the three-dimensional structure of this mutant homo-hexadecamer should provide significant insight into the molecular mechanism of chaperonin-mediated protein folding. Here, we report the crystallization and preliminary X-ray characterization of this mutant archaeal group II chaperonin α-subunit and the initial phasing for the crystal structure determination.

2. Material and methods

2.1. Protein purification

The expression vector for the chaperonin α-subunit from *T.* KS-1, including two amino-acid substitutions, was overexpressed in *E. coli* BL21(DE3) and purified by a previously reported method (Yoshida *et al.*, 2001). The protein solution was concentrated to approximately 30 mg ml^{-1} in buffer *A* (20 m*M* Tris–HCl pH 7.2, 10 m*M* $MgCl_2$) by ultrafiltration with Centricon-30 (Amicon). The protein concentration was estimated using a value of 0.37 mg ml^{-1} for A_{280}. The purity of the sample was analyzed by SDS–PAGE (13% acrylamide) and native PAGE (6% acrylamide).

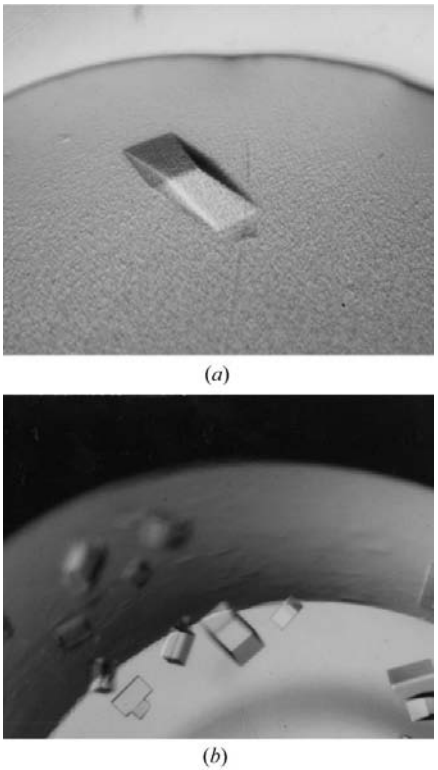

Figure 1
Crystals of the group II chaperonin α-subunit from *T.* KS-1. (*a*) Form I crystal with approximate dimensions of 0.15 × 0.15 × 0.4 mm; (*b*) form II crystals with approximate dimensions of 0.3 × 0.3 × 0.2 mm.

2.2. Crystallization

All crystallization experiments were carried out by the sitting-drop vapour-diffusion method at 293 K, employing 6–8 µl drops containing equal volumes of the protein solution and reservoir solution.

Crystallization conditions were performed for both the wild-type and the mutant (G65C/I125T) α-subunit. Crystals of the wild type were not obtained from any crystallization conditions, whereas crystals of the mutant appeared under two different conditions after random screenings; ammonium sulfate and polyethylene glycol 6000 were used as precipitants in conditions I and II, respectively. Long prismatic crystals (form I) with dimensions of 0.15 × 0.15 × 0.4 mm were obtained from condition I (100 m*M* Tris–HCl pH 8.0, 2.1 *M* ammonium sulfate and 50 m*M* potassium glutamate) (Fig. 1*a*). On the other hands, plate-like crystals (form II) with dimensions of 0.3 × 0.3 × 0.2 mm were obtained from condition II (100 m*M* Tris–HCl pH 8.0, 3.5% polyethylene glycol 6000 and 200 m*M* magnesium chloride) (Fig. 1*b*).

3. Results and discussion

The form I crystals diffracted X-rays at least to 2.4 Å resolution with synchrotron radiation at beamline BL6A of the Photon Factory, KEK, Japan (Fig. 2*a*). The wavelength, camera distance and oscillation range were 1.000 Å, 429.7 mm and 1.0°, respectively. However, the form II crystals only diffracted X-rays to 6 Å resolution (Fig. 2*b*). Therefore, diffraction data were collected using the form I crystals. Crystals soaked in a crystallization buffer with 20% glycerol as a cryoprotectant were flash-cooled with liquid ethane and all data collections took place at 100 K with a MSC cryosystem (Rigaku). The diffraction data were measured on a MAR CCD area detector at the BL41XU beamline, SPring-8, Harima, Japan. The wavelength, camera distance and oscillation range were 0.7085 Å, 250 mm and 0.5°, respectively. The crystals were found to belong to space group $P42_12$, with unit-cell parameters $a = b = 209.3$, $c = 156.1$ Å; diffraction extends to at least 2.4 Å. Assuming four subunits per asymmetric unit, the V_M value was calculated to be 3.6 Å^3 Da^{-1}, which falls into the range observed for most protein crystals (Matthews, 1968). Diffraction intensities in each image were integrated and merged with the programs *MOSFLM* and *SCALA* (Collaborative Computational Project, Number 4, 1994). The data-processing statistics are summarized in Table 1.

The polyalanine model of a monomeric α-subunit from *Thermoplasma acidophilum* was used as a search model in the molecular-replacement method. Although each step gave no clear solution, the high non-crystallograhic symmetry in the group II chaperonin (an eightfold axis and eight twofold axes perpendicular to the eightfold axis) was useful in revealing four real solu-

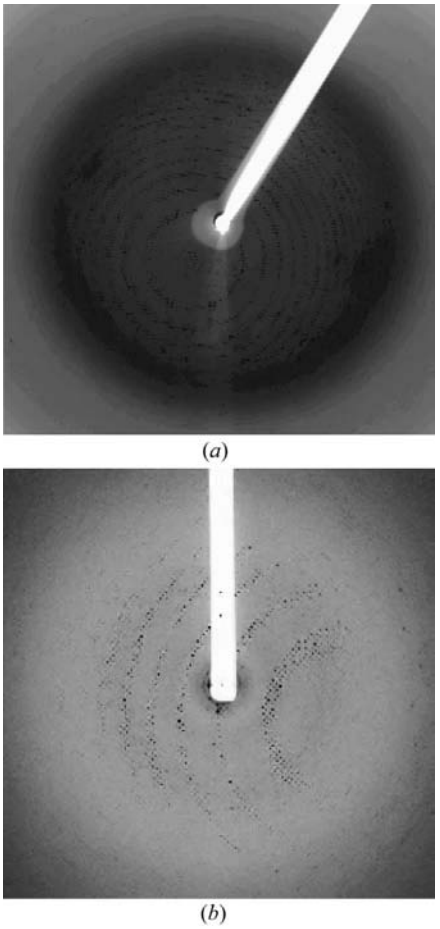

Figure 2
X-ray diffraction patterns from crystals of the group II chaperonin α-subunit from *T.* KS-1. (*a*) Form I collected at BL41XU (SPring-8, Harima, Japan); (*b*) form II collected at BL-6A (Photon Factory, KEK, Japan).

Table 1
Data-processing statistics.

Values in parentheses are for the outer resolution shell.

Space group	$P42_12$
Resolution (Å)	20–2.4
No. of observations	611685
No. of unique reflections	131 293
Completeness (%)	99.3 (98.1)
R_{sym}† (%)	5.5 (42.6)
$I/\sigma(I)$	11.7 (1.7)
Multiplicity	4.6 (3.3)

† $R_{sym} = \sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the ith observed intensity and $\langle I\rangle$ is the average intensity obtained from multiple observations of symmetry-related reflections.

tions. The 50 highest solutions from the direct rotation function in *X-PLOR* (Brünger, 1992) were filtered by PC-refinement. The first and second or the third and fourth solutions from PC-refinment were approximately related by a 45° rotation along the *c* axis (Table 2*a*). The object positions of the four highest solutions from the PC-refinement were searched with the translation function in *X-PLOR*. All four PC-refinement solutions gave only special positions with respect to the *xy* plane (*e.g.* the result from the first PC-refinement solution is shown in Table 2*b*). This was expected as the eightfold axis of the chaperonin is consistent with the crystallographic fourfold axis in the cases of both *Thermoplasma acidophilum* and *T*. KS-1 and the origin of model was not moved prior to the molecular replacement. Finally, individual subunits were divided into five domains, which can be predicted by the sequence alignment, and rigid-body refinement was perfomed. Although the translation function did not show any significantly high correlation coefficients, the rigid-body refinement successfully reduced the *R* value by refining the molecules one by one at each refinement cycle (Table 2*c*). Further analysis and crystallographic refinement are now in progress.

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Table 2
Molecular-replacement statistics.

True solutions are highlighted in bold.

(*a*) Rotation-function statistics (10–4 Å).

No.	α (°)	β (°)	γ (°)	Rotation function
1	**17.3**	**7.6**	**37.6**	**0.0423**
2	**14.7**	**8.2**	**355.6**	**0.0411**
3	**18.8**	**7.1**	**11.5**	**0.0408**
4	**23.0**	**6.9**	**321.5**	**0.0403**
5	15.0	33.7	13.1	0.0401
6	55.7	11.8	42.0	0.0398

(*b*) Solutions of the translation function with rotation-function solution No. 1 (12–5 Å).

No.	*a*	*b*	*c*	Translation function
1	**0.008**	**0.489**	**0.000**	**0.087**
2	**0.008**	**0.489**	**0.500**	**0.087**
3	0.008	0.489	0.294	0.083
4	0.008	0.489	0.008	0.082

(*c*) Rigid-body refinement statistics (20–4 Å).

	R_{work} (%)	
	Initial	Final
Solution 1†	54.4	53.9
Solution 1 + 2	53.8	52.3
Solution 1 + 2 + 3	53.7	52.8
Solution 1 + 2 + 3 + 4	51.8	49.0

† Solution numbers correspond to those of the rotation function.

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