X-ray crystallographic studies of group II chaperonin from hyperthermophilic archaea

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Introduction

Chaperonins, ubiquitous folding chambers for newly synthesized or denatured proteins, have been divided into two groups. GroEL from *Escherichia coli*, whose properties have been well studied, is a member of group I chaperonins, and the chaperonins from other species of eubacteria and endosymbiotic organelles also belong to the group I. On the other hand, group II chaperonins exist in archaebacteria, and the eukaryotic cytosl. As a one of major differences between two groups, group I chaperonins requires a single ring cofactor (termed as GroES in *Escherichia. coli*) for enclosing its cavities, while group II chaperonins have built-in lid segment by itself, and no homologue of the cofactor has been found. Moreover, most of the group I chaperonins consist of double homo-heptameric ring whereas group II chaperonins are found in archaea and cytoplasm of eukaryotes. Up to now, there is less knowledge about the mechanism of group II chaperonin.

The G65C/I125T mutant of α subunit hexadecamers of the group II chaperonin from *Thermococcus* strain KS-1 shows activities for ATP hydrolysis and capturing denatured proteins, but is unable to refold them in ATP depending manner, although the wild type of the recombinant α hexadecamer of this chaperonin has all three activities.

Materials and Method

α subunit hexadecamer from *Thermococcus* KS-1 was overexpressed in *E.coli*, purified and crystallized as described [1]. Diffraction data was collected at 100 K at the Photon Factory (BL6A and BL6B). Initial phases were obtained with the molecular replacement using the previously determined crystal structure of α subunit of archaeal chaperonin from *Thermoplasma acidophilum* as a model. After the crystallographic refinement using the program CNS, R_{work} and R_{free} were reduced 26.8 % and 28.9 %, respectively.

Results and Discussion

The crystal structure of mutant (G65C/I125T) α chaperonin from *Thermococcus* KS-1 was determined at 2.8 Å resolution by the molecular replacement method. The overall structure is composed of two stacked rings, each of which is comprised of eight α subunits, with the height of 156 Å and the diameter of 159 Å. Four α subunits are included in an asymmetric unit with the space group $P42_12$. Of these four α subunits, two consist in one ring, and the others two are in the opposite ring. The 8-fold axis in the hexadecamer of the chaperonin is consistent with a crystallographic 4-fold axis.

The whole structure has a spherical aspect and entrances of the cavities are completely closed with hydrophilic environment providing into the cavity, which is considered as a refolding active form for denatured proteins. This result seemed to be strange, since this mutant had already been shown to defects refolding activities *in vitro*. But, comparison of the present structure with the GroEL structure revealed that substituted residue (G65C) should affect on the conformational rearrangement for a refolding cycle. Namely, the side chain of cystein at the 65th position is too balky to allow the chaperonin to undergo local conformational changes, which lead drastic conformational change later in the course of the ATP-depending cycle.

References

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