Biological Science

5A, 6A/2008G141

Crystal structure of the C-terminal peptide-binding domain of human Hsp40

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Introduction

The molecular co-chaperone Hsp40 interacting with Hsp70 plays important roles in cellular processes including protein folding, assembly, degradation, and suppression of the non-native protein aggregation[1]. During protein refolding, the N-terminal DnaJ-like domain of type II Hsp40 stimulates the ATPase activity of Hsp70. C-terminal peptide-binding domain of Hsp40 (Hsp40-CTD) interacts with non-native polypeptides and also with the C-terminal region of Hsp70, and delivers the polypeptides to Hsp70[2]. In this study aiming at elucidation of co-chaperone mechanism of Hsp40, we determined the crystal structure of human Hsp40-CTD (Asp161−Ile340).

Materials and Method

Crystals of peptide-free Hsp40-CTD was obtained as a monoclinic $P2_1$ form by the hanging-drop vapor diffusion method using PEG3350 as a precipitant. For phasing, an Au-derivative crystal was prepared by soaking Hsp40-CTD crystals in the reservoir solution containing $KAu(CN)_4$. Diffraction data sets were collected from flash-frozen crystals at Photon Factory BL-5A and 6A.

Data sets were processed and scaled using *HKL2000*. Initial phases were obtained with the single isomorphous replacement method combined with anomalous scattering effects. Two Au binding-sites per asymmetric unit were found by interpreting the isomorphous and anomalous difference Patterson maps. The phases were improved through solvent-flattening, averaging, and histogram matching. The crystal structure of the Hsp40-CTD was refined at 1.85 Å resolution.

Results and Discussion

Hsp40-CTD in an asymmetric unit of the crystal exists as a twisted, horseshoe-shaped homo-dimer in which each subunit is related to the other by a non-crystallographic two-fold axis. The first five residues in the N terminus of each subunit are not visible in the electron-density map. The residues 186−203 and 223−232 of subunit B unlike of A are also not visible. These are judged as flexible regions. The subunit consists of eleven β-strands (β1−β11) and three α-helices (α1, α2, and α3), and is folded into three regions: elongated globular domains I (residues 165−241) and II (250−320), and a C-terminal helix region (321−340). Domains I and II show a common topology, however the domain II has an extra short β-strand β6. Domain I consists of a three-stranded β-sheet (β1, β4, and β5), two-turn α-helix (α1), and two-stranded anti-parallel β-sheet (β2 and β3). Domain II consists of a four-stranded β-sheet (β6, β7, β10, and β11), two-turn α-helix (α2), and two-stranded anti-parallel β-sheet (β8 and β9). The dimeric interface is formed through hydrophobic interactions between the C-terminal helix regions and also between the α2 helices in domains II.

A hydrophobic groove of about 20 Å in length and 15 Å in width is noticed on the trunk of domain I. This groove spanned between Thr180 and Ile235. Positively-charged lysine residues are located near the groove. It is presumed that the negatively-charged EEVD motif in the C-terminal region of Hsp70 is recognized by the groove with the positively-charged residues in its vicinities.

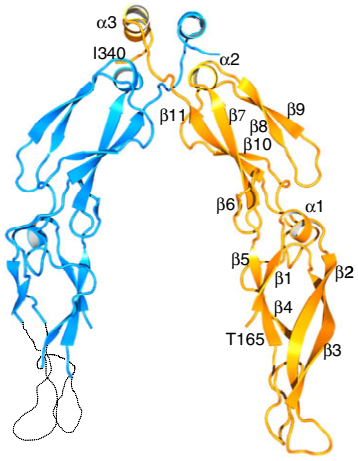

Figure Crystal structure of human Hsp40-CTD. The subunit A and B are shown in orange and cyan, respectively. The missing region is indicated by dotted line.

References

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