Biological Science

5A, 6A, 17A/2008G141

# Crystal structure of the C-terminal peptide-binding domain of human Hsp40 complexed with a C-terminal octapeptide of human Hsp70

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## Introduction

The molecular chaperone Hsp40 and Hsp70 play 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 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 type II Hsp40-CTD (Asp161-Ile340) and its complex with a C-terminal octapeptide of human Hsp70, GPTIEEVD (residues 634′-641′).

## Materials and Method

Crystals of Hsp40-CTD complexed with the octapeptide were obtained as a monoclinic $P2_1$ form by hanging-drop vapor diffusion method using PEG3350 as a precipitant. Diffraction data sets were collected from flash-frozen crystals at Photon Factory BL-5A, 6A, and 17A. Data were processed and scaled using *HKL2000*. The crystal structure of the complex that is isomorphous to the previously determined peptide-free Hsp40-CTD structure was refined at 1.85 Å resolution.

The atomic coordinate and diffraction data of this crystal structure have been deposited under accession number 3AGY.

## Result 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 local two-fold axis. The subunit consists of eleven β-strands (β1-β11) and three α-helices (α1, α2 and α3), and is folded into three regions: long globular domains I (residues 165-241) and II (250-320), and a C-terminal helix region (321-340). The dimeric interface is formed through hydrophobic interactions between C-terminal helix regions and also between the α2 helices in domains II.

The octapeptides are located in two sites, 1 and 2, of domains I. The octapeptide bound to site 1 forms anti-parallel β-sheet with the β2 strand. The negatively charged side-chains of Glu638′, Glu639′, and Asp641′ form salt bridges with the side-chains of Lys residues. The side-chain of Ile637′ fit into the hydrophobic concave formed by Met183, Ile185, Ile235, and Phe237. The octapeptide bound to site 2 form anti-parallel β-sheet with the β4 strand. The negatively charged side-chains of Glu638′, Asp641′, and the C-terminal carboxyl group form salt bridges with the side-chains of Lys residues. The side-chains of Pro635’ and Ile637’ interact with the surface-exposed hydrophobic region of domains I.

These results indicate that Hsp40 has two peptide-recognition sites both for the Hsp70 C-terminus. The site 1 is attributable to the major recognition site toward the Hsp70 since the extent of the electrostatic interactions and the atomic contacts between Ile637’ and the concave is larger than site 2. It is conceivable that the site 2 is the peptide-recognition site toward non-native polypeptides, because the hydrophobic region of the site 2 is wider and shallower than that of the site 1 and polypeptides having bulkier hydrophobic side-chains could be bound to this region.

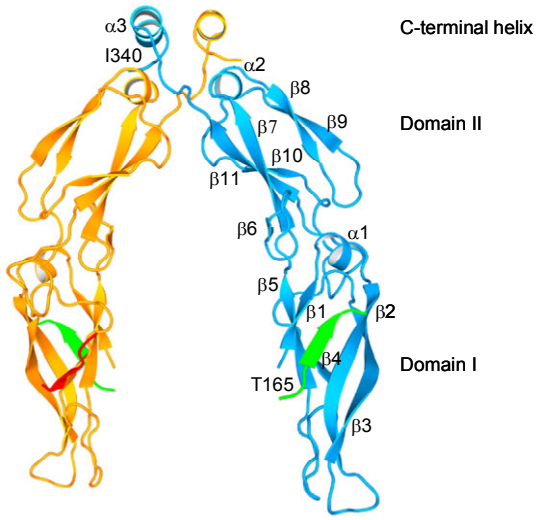


**Figure** Structure of human Hsp40-CTD complexed with a C-terminal octapeptide of Hsp70. The octapeptides bound to site 1 and 2 are shown in green and red, respectively.

## References

[1] Hartl, F. U. & Hayar-Hartl, M. *Science*. 295**,** 1852 (2002).
[2] Han, W. & Christen, P. *J. Biol. Chem*. 278, 19038 (2008).

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