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Crystal structures of the mutant of human Hsp40, Hdj1, C-terminal peptide-binding domain 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 interacts with non-native polypeptides and also with the C-terminal region of Hsp70, and delivers the polypeptides to Hsp70[2]. Previously, we determined the crystal structure of human type II Hsp40, Hdj1$_{161–340}$ complexed with a C-terminal octapeptide of human Hsp70, GPTIEEVD (residues 634-641) (PDB code 3AGY and 3AGZ) [3,4]. In the structures, Hdj1$_{161–340}$ exists as a twisted, horseshoe-shaped homodimer in which each protomer is related to the other by a local two-fold axis. The octapeptides are bound to two distinct sites (site 1 and 2) of each protomer. These two sites are located back to back with each other within a protomer. To establish the roles of these two sites, it is necessary to carefully design Hdj1 mutants in which one site is disrupted while the other remains unchanged, and to determine their co-chaperon activity. In this study aiming at elucidation of co-chaperone mechanism of Hsp40, we determined the crystal structures of two Hdj1$_{161–340}$ mutants at these sites complexed with the octapeptide.

Materials and Method

Site 1 (K182A) or site 2 (T216K) mutants of Hdj1$_{161–340}$ complexed with the octapeptide were crystallized at 293 K or 277 K by hanging drop vapor diffusion method using PEG3350 as a precipitant. The crystals were obtained as a monoclinic $P2_1$ form which is isomorphous to the previously determined wild type Hdj1$_{161–340}$ crystal (PDB code 3AGX). Diffraction data sets were collected from flash-frozen crystals at Photon Factory BL-5A and AR-NE3A. Data were processed and scaled using *HKL2000*. Crystal structures of the mutant Hdj1$_{161–340}$ complexed with the octapeptide were refined at 1.95 Å and 2.00 Å resolution, respectively.

Result and Discussion

In the wild type Hdj1$_{161–340}$ complex (3AGY), Lys 182 side-chain forms salt bridge with the C-terminal carboxyl group of Asp 641. In the structure of site 1 mutant K182A, the octapeptides were bound to both sites 1 and 2 as observed in 3AGY structure. However, the salt bridge is lost due to the mutation to Ala, which may weaken the affinity toward the octapeptide at site 1.

Thr 216 at site 2 is one of the residues forming hydrophobic surface interacting with the side-chains of Pro 635 and Ile 637 of the octapeptide. In the structure of site 2 mutant T216K, the octapeptides were bound only to site 1 with the same binding mode observed in 3AGY structure. The absence of the bound octapeptide at site 2 could be ascribed to the steric hindrance caused by the mutation to Lys residue.

These structures suggest that K182A and T216K mutations reduce the affinity toward the octapeptide at sites 1 and 2, respectively. Determination of the co-chaperon activity of the intact Hdj1 with one of these mutations is in progress. This would reveal which site is (or both site are) essential for the co-chaperon activity.

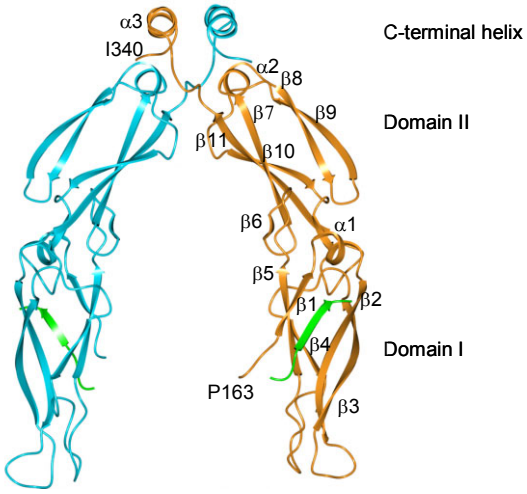

Figure Structure of human Hdj1$_{161–340}$ T216K complexed with a C-terminal octapeptide of Hsp70. The octapeptides bound to site 1 are shown in green.

References

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