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Structure of the free form of the N-terminal VH1 domain of monomeric α-catenin

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ABSTRACT

The N-terminal vinculin-homology 1 (VH1) domain of α-catenin facilitates two exclusive forms, a monomeric form directly bound to β-catenin for linking E-cadherin to F-actin or a homodimer for the inhibition of β-catenin binding. Competition of these two forms is affected by ~80 N-terminal residues, whose structure is poorly understood. We have determined the structure of the monomeric free form of the αN-catenin VH1 domain and revealed that the N-terminal residues form α1 and α2 helices to complete formation of the N-terminal four-helix bundle. Dynamic conformational changes of these two helices control formation of the β-catenin-bound monomer or unbound homodimer.

1. Introduction

The cytoskeletal protein α-catenin plays a key role in establishing and maintaining adherens junctions (AJs) [1–3]. At the cytoplasmic side of AJs, E-cadherin binds β-catenin and β-catenin binds α-catenin that binds actin filaments (F-actin) [4–6]. α-Catenin contains three conserved vinculin-homology regions, referred to as VH1, VH2 and VH3 [7] (Fig. 1a). As found in the vinculin structures, which consist of α-helix bundles A–H [8,9], recent X-ray studies have shown that near full-length α-catenin in the dimeric form comprises multiple helix bundles [10,11]. However, these studies failed to provide details of the intra-molecular interactions for a complete understanding of α-catenin function due to the limited resolution (3.7 Å or 6.5 Å). The N-terminal VH1 domain of α-catenin, which is also referred to as the N-terminal (N) or dimerization domain (DD), encompasses the VH1 region to form two α-helix bundles, A and B, and mediates homo-dimerization or hetero-dimerization by binding to β-catenin, exclusively [10–12] (Fig. 1a). In the homodimeric form, N-terminal helix bundle A contains only two α-helices that form an intermolecular four-helix bundle with the other α-catenin protomer for dimerization. In the β-catenin-bound form, however, the N-terminal bundle contains three α-helices to complete formation of a four-helix bundle with an α-helix from β-catenin. Thus, the N-terminal VH1 domain plays a pivotal role in the functional switch of α-catenin. Furthermore, recent studies have shown an important role of the VH1 domain in interactions with non-cytoskeletal proteins such as LIM domain-containing protein Ajuba [13], ERM protein merlin [14] and Hippo signaling protein Yap/14-3-3 [15]. Interestingly, α-catenin possesses a conformationally flexible N-terminal region comprising ~80 residues, and removal of these residues stabilizes the homodimer. Since the monomer, unlike the dimer, binds β-catenin, the nature of the N-terminal residues is critical for establishing molecular switch functionality. Unfortunately, the flexible N-terminal ~80 residues were artificially truncated for crystallization in the reported structures [10,12] or were not observed in the low resolution structure [11]. A recently reported structure at 2.8 Å resolution of an N-terminal 17-residue truncated VH1 domain of αN-catenin bound to a β-catenin fragment, hereafter referred to as the αN-β catenin complex, revealed that the N-terminal four-helix bundle contains one short extra α-helix (αN-catenin residues 21–36), which stabilizes β-catenin binding [16], implying a veiled function of the N-terminal residues. However, there is no crystal structure of the monomeric free form of the VH1 domain, which is preventing a complete understanding

Author contributions: T.S., Y.H. and T.H. conceived and designed the project. T.S. and Y.H. were responsible for construct design for protein preparation and subcloning. T.S. executed protein biochemistry, crystallization, data collection and solved and refined the complex structures, helped by Y.H. T.S. performed binding studies with mutant studies, as well as circular dichroism measurements. T.S. Y.H. and T.H. interpreted data, and T.S. and T.H. wrote the manuscript.

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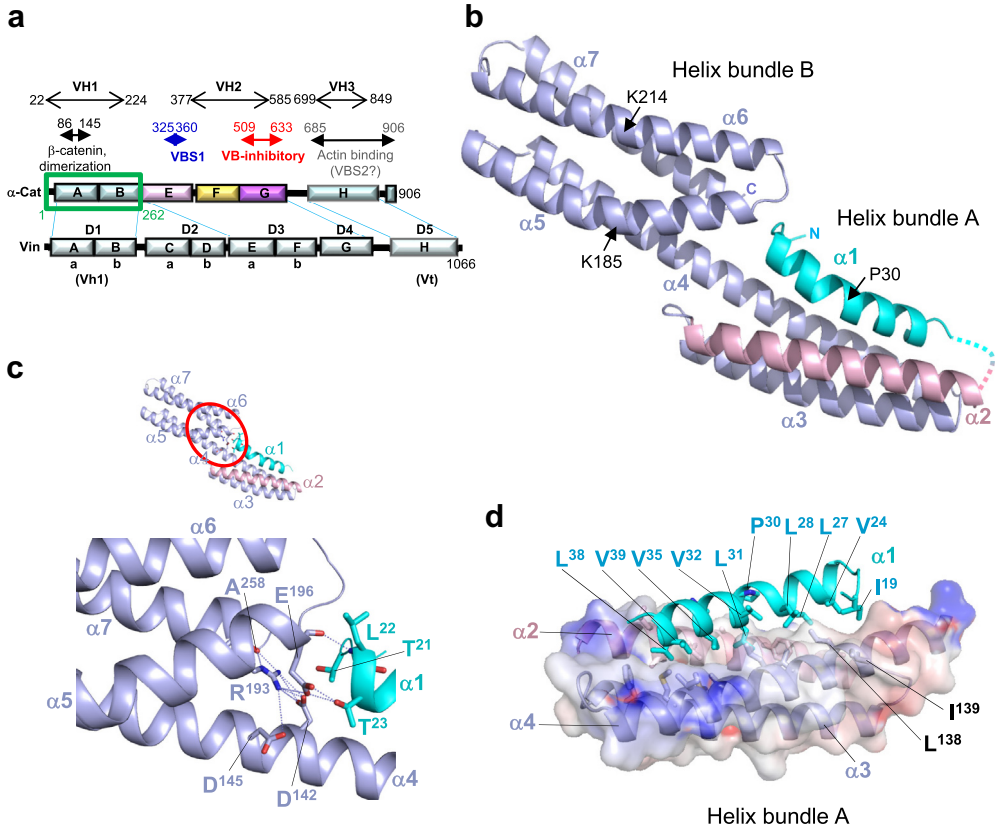

Fig. 1. Structure of the monomeric VH1 domain of αN-catenin. (a) Domain organization of α-catenin and vinculin. Four regions of α-catenin correspond to six out of eight helix bundles (A–H) of vinculin, which comprises five domains (D1–D5). The residue numbering for αE-catenin is given. M-fragment encompasses helix bundles F and G. (b) The overall structure of the VH1 domain of αN-catenin of the present study is represented as a ribbon model. Two helix bundles, A and B, are formed with N-terminal α1 helix (cyan), α2 helix (magenta) and five other helices (blue). Three helix kinks (α1, α5 and α6 helices) are indicated by arrows with the residue at the kink. Seventeen N-terminal residues (residue numbers 1–17) and 12 residues (residues 42–53 as represented by dotted lines) forming α1–α2 loop and the last C-terminal residue (261) are missing in the current model. (c) The contact sites between helix bundles A and B. α1 helix contacts helix bundle B with bifurcated hydrogen bonds between Thr23 and Glu196 of α5 helix and a main chain–main chain hydrogen bond. Salt bridges formed between Arg193 (from α5 helix) and Asp142 and Asp145 (from α4 helix) also stabilize the bundle orientation. Hydrogen bonds are indicated with dotted lines. (d) Helix bundling of α1 helix with the rest of helix bundle A is shown as a transparency surface representation and ribbon model. At the interface of the nonpolar residues (side chains as stick models) α1 helix is docked into the hydrophobic groove formed between α2 and α4 helices. The side chain of Ile19 at the N-terminal end of α1 helix makes nonpolar contacts with Leu138 and Ile139 from α4 helix.

of this important protein. Here, we report on the crystal structure of the VH1 domain of αN-catenin in the free form and reveal the complete four-helix bundle structure with two α-helices (α1 and α2) formed by the N-terminal residues. We identified key residues stabilizing this helix association with the rest of the helix bundle.

2. Materials and methods

2.1. Protein preparation

Each pET-47b [+] vector (Novagen) carrying a DNA fragment encoding mouse αN-catenin (1–261) or αE-catenin (1–263) was transformed into *Escherichia coli* strain BL21Star (DE3) (Invitrogen) cells for protein expression. Protein expression was performed at 20 °C in Luria–Bertani medium containing 0.1 mM isopropyl-β-D-thiogalactopyranoside. The proteins were purified using a Ni-NTA resin (Qiagen), cation-exchange (HiTrap SP HP, GE Healthcare) and gel filtration (Superdex 75 pg, GE Healthcare) chromatography at 4 °C. The monomeric and dimeric forms were separated using size-exclusion chromatography (SEC). In our SEC experiments, αN-catenin VH1 domain (1–261) gave a dominant peak (80–90%) for the monomer with a small peak (10–20%) for the dimer, while αE-catenin VH1 domain gave a smaller peak (~35%) for the monomer with a larger peak (~65%) for the dimer. The purified proteins were verified using matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI–TOF MS; Bruker Daltonics).

2.2. Crystallization, data collection and structure determination

Crystallization screening was performed at 4 °C utilizing HYDRA II (Art Robbins) or Mosquito LCP (TTP Labtech) with commercial crystallization screening kits. The best crystals of αN-catenin (1–261) were generated from a solution obtained by mixing 1.0 μl protein solution (20.8 mg/ml, 0.7 mM protein) and 1.0 μl precipitant solution containing 0.1 M Bis–Tris propane (pH 6.5), 0.2 M NaI and 19–21% (w/v) PEG3350. Crystals were flash-frozen (25% ethylene glycol) and served for X-ray diffraction data collection at 100 K using a Rayonix MX300HE detector and beamline BL44XU at SPring-8, Harima, Japan. All data were processed with the HKL-2000 program suite [17] (Table 1). Phases were determined by molecular replacement (MR) using the program PHASER [18] with the dimer of αE-catenin as a search model (PDB ID 1DOV). Models were refined through alternating cycles of Coot [19], CNS [20] and PHENIX [21] programs to 2.5 Å resolution. No outliers were flagged in the Ramachandran plots using MolProbity [22]. The refinement statistics are summarized in Table 1. Structural superposition was performed using the program SUPERPOSE [23] and molecular illustrations were prepared using the program PyMOL (DeLano Scientific, CA).