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^1H , ^{13}C and ^{15}N resonance assignments of the N-terminal domain of Vta1-Vps60 peptide complex

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Abstract

Vta1 and Vps60 are two ESCRT associated proteins, their direct interaction enhances Vps4 ATPase activity. The N-terminal domain of Vta1 (residues 1–167aa, named as Vta1NTD) contains two tandem MIT domains, which specifically recognize Vps60 and Did2 but not other ESCRT-III subunits. The fragment Vps60 (128–186aa) was reported to display full activity of Vps60, which stimulates Vps4 ATPase in a Vta1-dependent manner. To study the structural basis for the interaction between Vta1 and Vps60, as a first step, here, we report the resonance assignments of the sequential backbone atoms and the side chains of the residues in the two components of Vta1NTD/Vps60_{128–186} complex at pH 7.0 and 20 °C (BMRB No. 18521).

Keywords

Vta1; Vps60; ESCRT; Vps4; MIT domain

Biological context

The multivesicular body (MVB) sorting pathway plays critical roles in several eukaryotic cellular processes (Piper and Katzmann 2007). Proper function of the MVB sorting pathway requires reversible membrane association of the endosomal sorting complexes required for transport (ESCRT) proteins, a process catalyzed by Vps4 ATPase (Babst et al. 1998). Vta1 acts as a positive regulator of Vps4 by stimulating its assembly and ATPase activity (Azmi et al. 2006). Different from Vta1, Vps60 does not bind to Vps4 directly, and it only stimulates Vps4 in a Vta1-dependent manner (Azmi et al. 2008); however the mechanism of the allosteric regulation by Vps60 is still unclear.

The crystal structure of the N-terminal domain of Vta1 (Vta1NTD) in free form reveals that it has two previously unknown tandem microtubule interacting and transport (MIT) domains within the structure (Xiao et al. 2008). These two domains mediate the interaction between Vta1 and the ESCRT-III proteins Vps60 and Did2. It was reported that the N-terminal of Vta1 mainly interacts with the fragment of the residues 128–186 of Vps60 (Vps60_{128–186}) (Azmi et al. 2008). However, the residues within the MIT domains of Vta1NTD perceived to be important for ligand binding based on their structural homology to the MIT domain of Vps4 are completely dispensable for the binding activity of Vta1 (Xiao et al. 2008). It thus appears that the interaction between the MIT domain of Vta1 and its binding partner utilizes a mechanism different from that of Vps4. To unravel this novel recognition mode of the MIT domain, here, we carried out our NMR experiments on the Vta1NTD/Vps60_{128–186} peptide complex.

Methods and experiments

The N-terminal domain of Vta1, amino acids 1–167 (Vta1NTD), and Vps60 fragment (amino acids 128–186) were amplified and cloned into a modified pET28b vector with a SUMO protein inserted between a His₆-tag and the Vta1NTD or Vps60_{128–186} coding region via *Bam*H I and *Xho* I restriction. The proteins were expressed in the *Escherichia coli* BL21 (DE3) strain grown in LB medium. For isotope labeling, M9 Minimal medium was used supplemented with ¹⁵NH₄Cl (Cambridge Isotope Laboratories) or ¹⁵NH₄Cl and ¹³C-glucose (Cambridge Isotope Laboratories). Protein expression was induced (12 h, 20 °C) with 0.4 mM IPTG (OD₆₀₀ = 0.8). Cells were harvested and resuspended in lysis buffer (50 mM Tris pH 8.0, 300 mM NaCl, 5 mM β-mercaptoethanol) and lysed with 10 μg/mL PMSF by sonication on ice. Lysates were clarified by centrifugation (30 min., 16,000 rpm), and the soluble proteins were purified by nickel affinity chromatography (G.E Inc) through using a linear gradient of 0–250 mM imidazole in the lysis buffer. Protein fractions were collected and dialyzed two times at 4°C into the lysis buffer. The His₆-SUMO tag was removed by incubation with ULP1 protease followed by a second nickel column. The fractions containing Vta1NTD or Vps60_{128–186} were pooled together, exchanged with the buffer (20 mM Tris-HCl, 50 mM NaCl, 1 mM dithiothreitol, pH 8.0), followed by loading them into anion exchange Resource Q chromatography (G.E. Inc). The pure protein fractions were eluted by using a linear gradient of Tris-HCl buffer, and verified by running SDS-PAGE gel and electrospray mass spectrometry. The detailed purification steps will be published elsewhere.

Two NMR complex samples were prepared in NMR buffer (25 mM sodium phosphate pH 7.0, 100 mM NaCl, 5 mM dithiothreitol-d₁₀ (DTT), 0.02% NaN₃). One is 1.5 mM uniformly labeled ¹⁵N-/¹³C-labeled Vta1NTD mixed with 1.8 mM unlabeled Vps60_{128–186}, the other is 1.5 mM uniformly labeled ¹⁵N-/¹³C-labeled Vps60 in complex with 1.8 mM unlabeled Vta1NTD. The mole ratio (1:1.2) used here was designed based on the binding affinity results from isothermal titration calorimetry (ITC) assay (which will be described elsewhere), and to keep ¹⁵N-/¹³C-labeled component in each complex in bound states. NMR experiments were conducted at 20 °C on a Varian Unity Inova 600 NMR spectrometer or on a Bruker Avance III-800 MHz NMR spectrometer, both equipped with cryoprobe, three or four channels and z-axis pulsed-field gradient. The standard suite of experiments for

assigning the ^1H , ^{13}C and ^{15}N backbone and side chain chemical shifts, and for the collection of NOE-based distance restraints were measured (Bax and Grzesiek 1993; Clore and Gronenborn 1998), which consists of the 2D ^{13}C -edited HSQC in both aliphatic and aromatic regions, and ^{15}N -edited HSQC; the 3D HNCA, HNCO, HN(CO)CA, HNCACB, CBCA(CO)NH, ^{15}N -resolved HSQC-TOCSY, HCCH-TOCSY in both aliphatic and aromatic regions, ^{15}N -resolved HSQC-NOESY, ^{13}C -resolved HSQC-NOESY for both aliphatic and aromatic resonances, 2D $(\text{H}\beta)\text{C}\beta(\text{C}\gamma\text{C}\delta)\text{H}\delta$ and $(\text{H}\beta)\text{C}\beta(\text{C}\gamma\text{C}\delta\text{C}\epsilon)\text{H}\epsilon$ spectra for correlation of $\text{C}\beta$ and $\text{H}\delta$ or $\text{H}\epsilon$ in aromatic ring used in aromatic protons assignment (Yamazaki et al. 1993). All spectra were processed with NMRPipe (Delaglio et al. 1995) and analyzed with Sparky 3. The ^1H chemical shifts of were referenced to 2,2-dimethylsilapentane-5-sulfonic acid (DSS), and the ^{13}C - and ^{15}N -resonances were indirectly referenced to DSS.

Assignments and data deposition

The sequential backbone and side chain resonance assignment had been deposited in the Biological Magnetic Resonance Data Bank (<http://www.bmrb.wisc.edu>) under the accession number 18521. The ^1H - ^{15}N -TROSY-HSQC spectrum of the (U- ^{15}N , ^{13}C) Vta1NTD/Vps60₁₂₈₋₁₈₆ complex sample is shown in Fig. 1, where the linewidth of each cross-peak seems to be narrow due to TROSY technique used in the experiment and slightly higher contour level of the spectrum. All the amide signals were observed with the exception of the amide signals of Met1, Glu141 and Leu161 of Vta1NTD. All the $\text{C}\alpha$ were assigned, while about 96% and 97% of the $\text{C}\beta$ and CO were assigned, respectively. ^1H and ^{13}C chemical shifts were obtained for approximately 98% of the CH_n and aromatic side chains. In 3D HNCO, the cross peak at ^{15}N 126.5 ppm, ^1H 7.0 ppm and ^{13}C 171 ppm was observed. However, only one cross peak (at ^{13}C 43 ppm) was found in the vertical strip of ^{15}N 126.5 ppm and ^1H 7.0 ppm in 3D HNCA, and no cross-peak was obtained at the same strip in 3D HN(CO)CA. Thus, in Fig.1, the signal at ^{15}N 126.5 ppm and ^1H 7.0 ppm was suggested to be from $\text{NH}\epsilon$ in Arg side-chain in Vta1NTD. In current study, its assignment could not be performed accurately.

In Fig. 2, the ^1H - ^{15}N -HSQC spectrum of the (U- ^{15}N , ^{13}C) Vps60₁₂₈₋₁₈₆/Vta1NTD complex is shown. The assignment of the labeled Vps60₁₂₈₋₁₈₆ peptide in complex with Vta1NTD was completed to 89.4% of the backbone resonances (CA, CB, C, N, H, HA, HB). The amide signals of Asp135, Met136, Gln137, Asp138, Glu139 and Met140 were not observed, reflecting a high degree of conformational flexibility in this region. Except this flexible region (residues 135–139), the assignment of side-chain resonances is 93.0% complete. Caused by the *cis-trans* isomerization of Pro186 in the C-terminus, two minor peaks of Thr184 and Leu185 were observed which were labeled with T184' and L185', respectively (Fig. 2). The Pro186 adopts a major *trans* conformation as demonstrated by the differences between their $\text{C}\beta$ and $\text{C}\gamma$ chemical shifts (Schubert et al. 2002)

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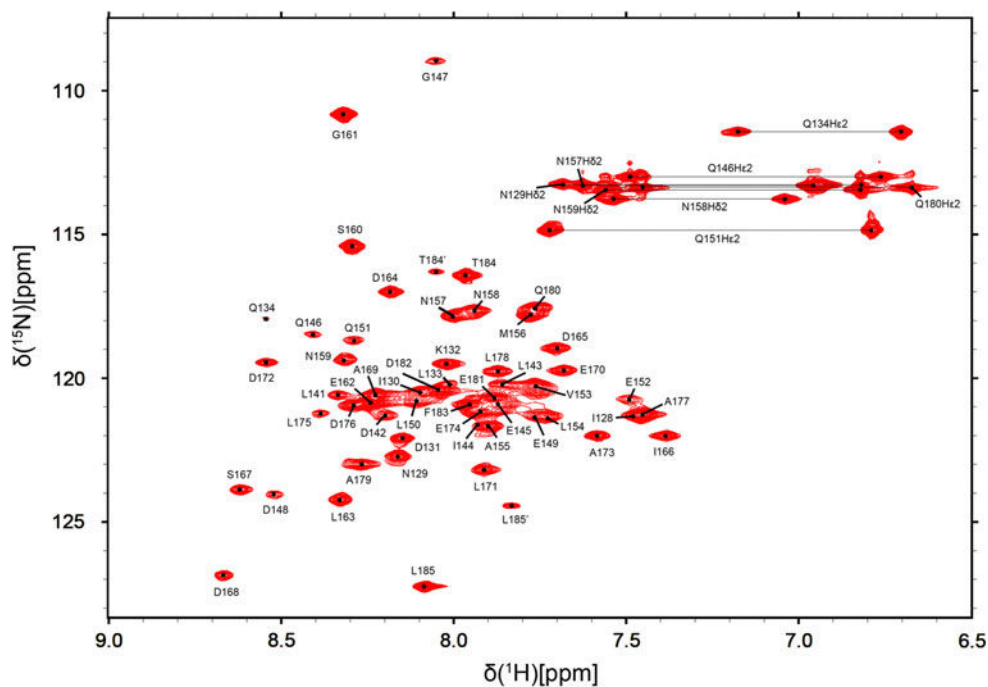


Figure 2. ^1H - ^{15}N -HSQC spectrum of the (U - ^{15}N , ^{13}C) Vps60₁₂₈₋₁₈₆/Vta1NTD complex measured at 20 °C at 600 MHz. Backbone resonance assignments are indicated in a one-letter amino acid code. Side-chain NH_2 amide resonances of Asn and Gln, connected by horizontal lines.