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Backbone and Side-chain NMR Assignments for the C-terminal Domain of Mammalian Vps28

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Abstract

Vps28 is one of four cytosolic proteins comprising the Endosomal Sorting Complex Required for Transport I (ESCRT-I). ESCRT-I is involved in sorting ubiquitinated proteins to multivesicular bodies (MVB) as well as in mediating budding of retroviruses. Here, we report the backbone and side-chain assignments of the mammalian C-terminal domain of Vps28 (mVps28^{CTD}), which is involved in interactions with other ESCRT components. We also compare the predicted secondary structures of mVps28^{CTD} with those of the published X-ray crystal structures of *Saccharomyces cerevisiae* and *Xenopus laevis* Vps28^{CTD}. These NMR resonance assignments will facilitate chemical shift mapping and structural determination of mammalian Vps28 interactions with other components of the endosomal sorting machinery that sorts ubiquitinated proteins for lysosomal degradation.

Keywords

Vps28; ESCRT; ubiquitin; lysosome

Biological context

The collective apparatus that sorts ubiquitinated proteins into intraluminal vesicles found in multivesicular/late endosomes is comprised of 4 Endosomal Sorting Complexes Required for Transport (ESCRTs): ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III (Hanson and Cashikar, 2012; Piper and Katzmman, 2007). These complexes are thought to assemble onto endosomal membranes in a sequential manner, driven by interactions with lipids, cargo, and protein-protein interactions between specific components of the different ESCRTs. ESCRT-0 recognizes ubiquitinated cargo, which in turn recruits ESCRT-I, then ESCRT-II, which nucleates the polymerization of ESCRT-III that completes the last steps of intraluminal vesicle formation and detachment from the limiting membrane of endosomes.

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Ethical Standards

All of our experiments comply with accepted ethical standards.

Conflict of Interest

The authors declare that they have no conflicts of interest.

In yeast, the C-terminal domain of Vps28 (Vps28^{CTD}) which is a component of ESCRT-I, binds to one of the Npl2-Zn-Finger (NZF) domains of the yeast ESCRT-II subunit, Vps36 (Bowers et al., 2004; Gill et al., 2007; Teo et al., 2006). This interaction is required for ESCRT assembly and function (Gill et al., 2007). Yeast Vps28^{CTD} also interacts with ESCRT-III, although how that interaction contributes to the biogenesis of multivesicular/late endosomes is unclear (Pineda-Molina et al., 2006). In contrast to yeast Vps36, mammalian Vps36 does not contain an NZF domain making it unclear as to how ESCRT-I interacts with ESCRT-II and potentially ESCRT-III (Im and Hurley, 2008). The 3.05 Å crystal structure of Vps28^{CTD} from *Sacharomyces cerevisiae* and the 1.3 Å crystal structure of *Xenopus laevis* Vps28^{CTD} reveal that Vps28^{CTD} is composed of a four-helical bundle (Gill et al., 2007; Pineda-Molina et al., 2006). Alignment of Vps28^{CTD} across different species highlights a conserved surface along helices 1 and 4, which lies on the opposite side of the yeast Vps28^{CTD} that binds the yeast Vps36 NZF domain (Pineda-Molina et al., 2006). These data suggest that there remain important interactions of the Vps28^{CTD} to be discovered. Here we report the backbone and side-chain assignments of mVps28^{CTD} and compare the secondary structures of mVps28^{CTD} predicted from these assignments with those of the X-ray crystal structure of yeast and toad Vps28^{CTD}.

Methods and experiments

Recombinant protein expression and purification

DNA encoding human Vps28^{CTD} (residues 124 – 221) was cloned into ampicillin resistance pET151D. The plasmid was transformed into BL21 DE3 *Escherichia coli* cells. Cells were grown in LB (Luria-Bertani) broth supplemented with 100 µg/mL of ampicillin at 37°C to an OD₆₀₀ of 0.67. The cells were then resuspended in ¹⁵N,¹³C-Spectra 9 minimal media supplemented with (20%) ¹⁵N,¹³C Celltone (Spectra Stable Isotopes) and grown one additional hour at 37°C prior to induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside.

The N-terminal 6xHis-tagged mVps28^{CTD} was purified over Talon resin. After cleaving off the His tag with TEV protease, the mVps28^{CTD} was purified by gel filtration over Superdex 200, and concentrated to 17 mg/mL in 40 mM Na₂PO₄, 50 mM NaCl, 3 mM NaN₃, 2 mM d₂erated DTT, pH 6.95. The NMR experiments were carried out by using 0.6 mM ¹⁵N,¹³C-labeled sample in this buffer in 10% D₂O/90% H₂O or 100% D₂O.

NMR spectroscopy

All spectra were acquired on a 500 or 800 MHz Bruker Avance II NMR spectrometer at 25 °C. A suite of triple resonance NMR experiments including HNCACB, HN(CO)CACB, HNCO, HN(CA)CO, HNCA, and HN(CO)CA experiments were collected for backbone assignments. Side-chain assignments were obtained by acquiring C(CO)NH, H(CCO)NH, HBHA(CO)NH, HCCH-TOCSY, ¹⁵N-NOESY, ¹⁵N-TOCSY, and ¹³C-NOESY spectra. The collected data were processed using NMRPipe (Delaglio et al., 1995) and analyzed with NMRViewJ (Johnson, 2010).

Resonance assignment and data deposition

Figure 1 shows that mVps28^{CTD} gave a well-dispersed ¹⁵N-¹H HSQC spectrum. All of the cross peaks present in this spectrum were assigned. For clarity, the more crowded region is enlarged in the inset. Excluding the N-terminal 7 residues of the tag used for affinity purification and 3 proline residues within Vps28^{CTD}, we obtained assignments for 95.7% of HN, 95.7% of N, 99% of C, and 100% of both Cα and Cβ. Excluding the aromatic side-chains and the Lys and Arg amines, 100% of the mVps28^{CTD} protons were assigned, 87.2% non-redundantly. The assigned chemical shifts were deposited in BioMagResBank (<http://www.bmrb.wisc.edu>) under the accession number 19569. The protein sequence of mVps28^{CTD} starts at residue N124 corresponding to the residue N8 in the deposited file.

Figure 2 shows the amino acid alignment of Vps28^{CTD} from human, mouse, the toad *Xenopus laevis*, and the yeast *Saccharomyces cerevisiae*. The human and mouse Vps28^{CTD} protein sequences are identical and show 7 amino acid differences to the *Xenopus laevis*Vps28^{CTD}. Figure 2 also shows a comparison of the secondary structures found in the determined crystal structures of yeast and toad Vps28^{CTD} compared to the secondary structure of mVps28^{CTD} predicted from our assigned NMR shifts using Talos+ (Shen et al., 2009), Dangle (Cheung et al., 2010), and d2D (Camilloni et al., 2012). Excellent agreement between these secondary structures was obtained, implying that the structure of the mammalian Vps28^{CTD} in solution used in this study is similar to the known crystal structures of Vps28^{CTD} from other species. Interestingly, M204 from helix α4 is strongly upfield shifted (Hβ at 1.263 and 0.597 ppm, Hγ at 1.441 and 0.708 ppm, and CεH₃ at 1.470 ppm) and gives strong NOEs to W183 that is located on helix α3 (Supplemental Fig. 1). These data indicate that M204 and W183 are located in close proximity, consistent with the known crystal structure (Supplemental Fig. 1). In summary, our NMR analyses suggest that the overall structure of mVps28^{CTD} in solution is similar to that observed in crystal structures, making it useful for mapping the protein:protein interactions using an NMR approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
 ^{15}N - ^1H HSQC spectrum of mVps28^{CTD}. The cross peaks are assigned and labeled. The inset shows the magnified view of a crowded region indicated by the dotted lines

Fig. 2.

Alignment of the amino acid sequence of Vps28^{CTD} from human, mouse, *Xenopus laevis*, and *Saccharomyces cerevisiae* with identical residues across species indicated in bold. Residues in the mVps28^{CTD} that differ from the *X. laevis* Vps28^{CTD} are underlined. Crystal structures of *X. laevis* Vps28^{CTD} (PDB ID: 2J9W) and *S. cerevisiae* Vps28^{CTD} (PDB ID: 2G3K) were used to map the regions of alpha helical segments (α1-α4) as shown in black rectangles. The alpha helical segments for mouse and human Vps28^{CTD}, which have identical sequences, were predicted from our assigned chemical shifts using the programs Dangle, δ2D, and TALOS+