Journal of Biomolecular NMR (2005) 31: 369–370
DOI 10.1007/s10858-005-0943-9
© Springer 2005

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C Resonance assignments and secondary structure determination reveal that the minimal Rac1 GTPase binding domain of plexin-B1 has a ubiquitin fold

Yufeng Tong* & Matthias Buck
Department of Physiology and Biophysics, Case Western Reserve University, Medical School, 10900 Euclid Avenue, Cleveland, OH 44106, U.S.A.

Received 20 October 2004; Accepted 06 January 2005

Key words: axon guidance, Rac1 binding, resonance assignment, secondary structure

Biological context

Growth, guidance and branching of axons are critical events that generate precise connections in the developing nervous system. The navigation of axons are directed by environmental cues, for example, protein ligands like semaphorins that are secreted or attached to target cells (Pasterkamp and Kolodkin, 2003). Similar signaling mechanisms are also used in other cellular processes, such as angiogenesis and cell motility. Plexins are a family of transmembrane receptors that interact with semaphorins through their extracellular domains (Tamagnone et al., 1999). On the intracellular side, the cytoplasmic domain of several members of the plexin family (e.g. -A1 and -B1) are known to directly bind to Rho-family small GTPases, Rac1 and Rnd1 (Vikis et al., 2000; Driessens et al., 2001). Rho-family GTPases remodel the cytoskeleton and are intimately involved in the motility and the positional maintenance of cells. Plexins are the first example of a receptor that signals directly between the guidance cues and Rho-GTPases, causing the attraction or repulsion of growth cones. However, the mechanism of this signal transduction remains to be understood in detail. No three-dimensional structures have been determined for the cytoplasmic domains of plexins to date.

We and others have found that the interaction of plexin-B1 with Rac1 or Rnd1 are confined to a small well structured cytoplasmic region (Vikis et al., 2000; Hu et al., 2001; Tong et al., 2005). The plexin-B1/Rac1 interaction has been shown to inhibit PAK activation (Vikis et al., 2002) and to enhance RhoA signaling (Hu et al., 2001). Very recently it was shown that upon binding of Rnd1 to the cytoplasmic region, plexin-B1 down-regulates R-Ras activity, functioning as a GTPase activating protein (GAP) (Oinuma et al., 2004). We have isolated the minimal Rac1 binding domain (RBD) of plexin-B1 using molecular biology and pull-down assays. Insight into the solution conformation of the RBD will help to understand the role of this domain in plexin-mediated signal transduction in mechanistic detail.

Methods and experiments

The gene encoding the minimal Rac1 binding domain (RBD, res. 1743–1862) of human plexin-B1 was subcloned into expression vector pET-11a. Two Lys residues were added to the N-terminal of the protein. The wild type RBD was found to exist as a weak homodimer in solution, giving an unfavorable distribution of line widths for NMR studies. Mutation W1830F monomerizes the protein without causing significant structural perturbation and has negligible effects on Rac1 binding affinity (Tong et al., 2005). Thus assignment was carried out on the W1830F mutant. Uniformly ^{13}C- or ^{15}N/^{13}C-labeled recombinant plexin-B1 RBD-W1830F was overexpressed in *E.coli* strain

*To whom correspondence should be addressed. E-mail: yufeng.tong@case.edu

BL21(DE3) in soluble form and purified. The purity of the protein was greater than 95% judged by SDS-PAGE. For assignment, 1.5–2 mM protein was exchanged into 25 mM phosphate buffer at pH 6.8, containing 50 mM NaCl, 4 mM DTT, 4 mM $MgCl_2$ in 90% H_2O/10% D_2O (for doubly labeled samples) or 99.9% D_2O (for ^{13}C-labeled samples). NMR experiments were carried out at 298 K either on a Varian Inova 600M Hz or on a Bruker Avance ICE600 spectrometer, both equipped with actively shielded z-axis gradient units. Proton chemical shifts (in ppm) were referenced relative to internal DSS. ^{15}N and ^{13}C references were set indirectly using the corresponding consensus ξ ratios. For backbone assignment, 3D HNCA, HNCACB, CBCA(-CO)NH, HNCO spectra were acquired. HNHA, H(CCCO)NH, C(CO)NH, HCCH-COSY, and HCCH-TOCSY were used for aliphatic side chain assignment. Reduced dimensionality experiments HBCB (CGCD)HD and ^{1}H-TOCSY-relayed HCH-COSY were carried out for assignment of aromatic side chains (Szyperski et al., 2002). All spectra were processed with nmrPipe and analyzed with NMRView.

Extent of assignment and data deposition

The ^{1}H–^{15}N HSQC spectrum of plexin-B1 RBD-W1830F closely matches that of the wild type protein at 10 μM acquired with a cryoprobe. At this concentration the wild type protein exists predominantly as monomer (Tong et al., 2005) and the correspondence of the spectra shows that mutation W1830F does not change the three-dimensional structure of the monomeric protein. Assignments of backbone $^1H^N$, ^{15}N, $^{13}C'$, $^{13}C^{\alpha}$, $^1H^{\alpha}$ resonances and of $^{13}C^{\beta}$, $^1H^{\beta}$ resonances are complete. Assigned amide group ^{1}H, ^{15}N resonances are shown in supplementary Figure 1a. In total, 96.7% of the non-labile protons have been assigned. For non-labile protons in the aliphatic side chains, only $^1H^{\delta}$ of P1798 is not assigned. The secondary structure of the domain was determined from consensus chemical shift index (Wishart and Sykes, 1994) (supplementary Figure 1b). While previously it was reported that this region of plexin is not homologous to any other protein with known structure, quite unexpectedly, we find secondary structure similarity and sequence homology between the plexin-B1 RBD and ubiquitin (supplementary Figure 1b). Preliminary structure calculations show that the domain has a ubiquitin fold. Recently, it was demonstrated that Rnd1 (a Rac1 homologue) and R-Ras bind to plexin-B1 simultaneously (Oinuma et al., 2004). The ubiquitin fold has been found in Ras binding proteins and its occurence in the Rac1 binding domain of plexin is intriguing. The ^{1}H, ^{15}N and ^{13}C chemical shifts of plexin-B1 RBD-W1830F have been deposited in the BioMagResBank under accession number 6346.

Supplementary material to this paper is available in electronic format at http://dx.doi.org/10.1007/s10858-005-0943-2.

Acknowledgements

We thank Dr. Alan Hall, UCL for providing a plasmid with the cytoplasmic region of plexin-B1 gene. Y.T. is the recipient of a postdoctoral fellowship from the American Heart Association (AHA), Ohio Valley Affiliate (0425678B). This work is partially funded by a Scientist Development Grant from the AHA, National Center (0335353N) to M.B.

References

Driessens, M.H., et al. (2001) *Curr. Biol.*, **11**, 339–344.
Hu, H., et al. (2001) *Neuron*, **32**, 39–51.
Oinuma, I., et al. (2003) *Science*, **30**, 862–865.
Pasterkamp, R.J., and Kolodkin, A.L. (2003) *Curr. Opin. Neurobiol.* **13**, 79–89.
Szyperski, T., et al. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 8009–8014.
Tamagnone, L., et al. (1999) *Cell*, **99**, 71–80.
Tong, Y., et al. (2005) *Structure* **13**, 7–15.
Vikis, H.G., et al. (2002) *Genes. Dev.*, **16**, 836–845.
Vikis, H.G., et al. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 12457–12462.
Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR* **4**, 171–180.