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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C backbone and side chain assignments of PSD-95 PDZ3 protein

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Biological context

As more is learned about the components that participate in cellular protein-protein interactions, many are increasingly being found to contain *protein interaction domains* (Pawson et al., 2003). These modular units are encoded within longer polypeptide structures, and facilitate the formation of selective, non-covalent interactions with other cytosolic or membrane-associated proteins. One such class is that of the *PDZ domain*, members of which are adept at recognizing their target protein partners primarily through the C-terminal residues of the latter. Among the most noted of the PDZ domain-containing proteins is the *postsynaptic density-95* protein (PSD-95, or SAP90). Localized at the neuronal synaptic junction – host to a number of proteins that mediate transient protein-protein associations – PSD-95 is involved in the control of synaptic strength and plasticity (Beique et al., 2003). Like other members of the MAGUK (membrane-associated guanylate kinase) class of proteins, PSD-95 is composed of several domain modules that facilitate binding to a variety of cellular proteins, primarily through the agency of its three PDZ domains (PDZ1, PDZ2, PDZ3) (Irie et al., 1997).

Both PDZ1 and PDZ2 have been structurally characterized by NMR (Tochio et al., 2000; Piserchio et al., 2002; Long et al., 2003) but PDZ3 has not. An X-ray crystallographic structure of PDZ3 in complex with a nine-residue peptide has been solved, but only four of the C-terminal residues of the ligand are sufficiently defined in the electron density to be accurately positioned. While the prominent molecular interactions have primarily been ascribed to the residues at the very C-terminus, those further along the chain do impact binding affinity (Songyang et al., 1997). An NMR-determined structure of PDZ3, in complex with an assortment of peptide ligands, will ideally clarify the nature of the interaction beyond the first few C-terminal residues. Further, the NMR solution structure will allow for investigation into the dynamic behavior of PDZ3 as a function of ligand binding. It will also serve as a starting point for structure-based ligand design, protein-ligand studies and rapid, NMR-based screening. The development of cell permeable inhibitory ligands for the PDZ domain will lead to their use as molecular probes to aid in the dissection of protein networks (Piserchio et al., 2004). The PDZ domains of PSD-95 may even prove to be biomedically viable targets, such as in the treatment of stroke-associated ischemic brain damage (Aarts et al., 2002).

Methods and experiments

PDZ3 was encoded as a GST-PDZ3 fusion in a pGEX-2T expression vector, which was used to transform *E. coli* BL21-Gold (DE3) (Stratagene). A single colony of cells bearing the GST-PDZ3 plasmid were cultured in 20 ml TB media with 50 μg/ml ampicillin at 37 °C for 12 h. The grown cells were centrifuged, resuspended and then incubated in 250 ml M9 mimimal media with unlabeled NH_4Cl and glucose (37 °C for 12 h). They were then harvested, resuspended and grown in 1 L M9 minimal media (50 μg/ml ampicillin) with 1 g/l $^{15}NH_4Cl$ (Cambridge Isotope Laboratories Inc. and Isotec), with or without 2 g/l $^{13}C_6$-D-glucose (Cambridge Isotope Laboratories Inc. and Isotec) at 37 °C. The culture was induced at 30 °C for 6 h with

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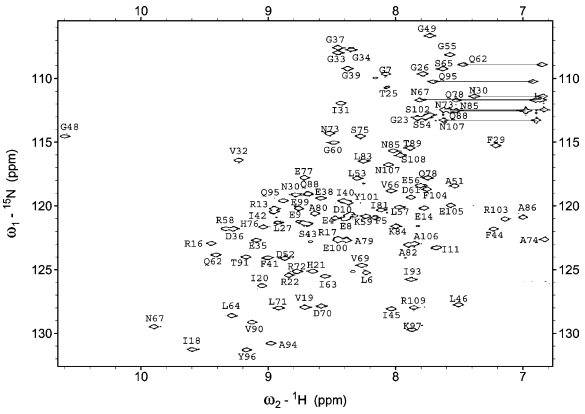

Figure 1. ^{1}H-^{15}N HSQC spectrum of PSD-95 PDZ3 (20 mM Na phosphate, 100 mM NaCl, pH 7.0, 20 °C) at 600 MHz. Horizontal lines designate side chain amide groups of asparagines/glutamines.

0.8 mM isopropyl β-D-thiogalactoside (IPTG). Harvested cells were resuspended in 1X PBS, 1% Triton-X and 19 mM β-mercaptoethanol before sonication. The lysate was centrifuged and the supernatant applied to a GST affinity column which was eluted with 3–4 volumes of 50 mM Tris-HCl with 5 mM reduced glutathione. The fusion was proteolytically cleaved at the thrombin recognition site with TPCK Trypsin (Pierce) at room temperature, followed by dialysis against 1X PBS with 1 mM DTT. The protein was then applied to a GST affinity column after which the sample was dialyzed against 20 mM Mes with 1 mM DTT and 10 mM NaCl (pH 6.0) for further purification by ion-exchange chromatography (Q HP Sepharose, Pharmacia) using NaCl gradients. Isolated PDZ3 samples were prepared for NMR by dialyzing them against 20 mM sodium phosphate, 100 mM NaCl (pH 7.0) buffer. Tryptic digestion and MALDI-TOF results confirm the overall size and sequence of the PDZ3 protein.

NMR experiments were performed at 20 °C on Bruker DMX 500 MHz and Varian INOVA 600 MHz spectrometers. The samples used corresponded to 90%/10% H_2O/D_2O solutions of either ^{15}N- or ^{13}C/^{15}N-labelled PDZ3 protein in the final phosphate/NaCl dialysis buffer (above). The following triple resonance 3D NMR experiments were conducted for the purpose of backbone assignments: HNCA, HNCOCA, HNCACB, HNCO and HNCOCA. Side chain assignments were accomplished via CBCA-CONH, HBHACONH, HCCH-TOCSY, Arg-H(CC)-TOCSY-NεHε, TOCSY-HSQC and NOESY-HSQC experiments. All spectra were processed using NMRPipe (Delaglio et al., 1995) and then analyzed using Sparky (T.D. Goddard et al., in preparation).

Extent of assignments and data deposition

PDZ3 protein contains 109 residues (101 plus 8 extra due to the construct). In Figure 1, nearly all the backbone NH resonances are observable and well-resolved. All the amide ^{1}H, amide ^{15}N and ^{13}Cα backbone resonances were assigned except for the NH resonance of residue S2 (which was unobservable). The S24-G26 NH resonances were either absent (S24) or quite weak, possibly reflecting the existence of chemical exchange effects in the S24-G26 region of this protein. Approximately 95% and 99% of the ^{13}C′ and ^{13}C$_\beta$ resonances were assigned, respectively. The assignments of more than 95% of the remaining ^{1}H side chain resonances have also been obtained.

The PSD-95 PDZ3 chemical shift assignments (^{1}H, ^{15}N, ^{13}C) have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6193.

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