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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of Gads C-terminal SH3 domain in complex with an RXXK motif-containing peptide derived from SLP-76

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Biological context

Gads is a hematopoietic-specific adaptor protein involved in signaling transduction downstream of the T cell receptor (Liu et al., 1999). It contains an SH2 domain flanked by two SH3 domain at the N- and C-termini. Gads interacts with the leukocyte protein of 76 kDa, or SLP-76, through its C-terminal SH3 domain, and is recruited to the linker of activated T cell, or LAT, via binding of its SH2 domain to a phosphotyrosine-containing motif in LAT upon TCR activation (Liu et al., 1999, 2001). The C-terminal SH3 domain of Gads has recently been shown to bind to SLP-76 by recognizing a novel peptide motif containing RXXK in SLP76 (Berry et al., 2002).

The interaction between Gads and SLP-76 is very strong with a dissociation constant, Kd, around 0.24 μM for the complex (Berry et al., 2002), compared to Kd's of 0.5–50 μM for typical SH3 domain-mediated interactions. In addition, the specificity of the Gads SH3 domain is unusual since conventional SH3 domains recognize a proline-rich core motif, PXXP, where X denotes any amino acids. A number of structures of SH3 domain exist in the literature, most of which in complex with PXXP-containing ligands that adopt the type-II polyproline (PPII) helical structure. To date, however, no structural information is available to account for the unique selectivity of the Gads C-terminal SH3 domain for RXXK-containing sequences.

We seek to determine the solution structure of the Gads C-terminal SH3 in complex with a peptide derived from SLP-76 containing the RXXK motif by NMR in order to understand the structural basis underlying the recognition of a non-canonical peptide motif by an SH3 domain. As a first step towards this goal, we report here the complete backbone and sidechain ^{1}H, ^{13}C, and ^{15}N assignments of the Gads C-terminal SH3 domain in complex with a peptide, APSIDRSTKPA, derived from its binding site in SLP-76.

Methods and experiments

A pGEX4T2 vector containing the Gads SH3-C domain as insert was used to transform *E. Coli* Uniformly ^{15}N/^{13}C-labeled Gads C-terminal SH3 domain was produced by growing the bacteria in a M9 medium supplied with $^{15}NH_4Cl$ and [$^{13}C_6$]-d-glucose (Cambridge Isopes). The GST-fused SH3 protein was purified on glutathione sepharose beads, and the SH3 domain was released by thrombin cleavage and further purified by passing through a Sephadex 75 (Amersham Pharmacia Biotech.) column on FPLC.

Due to the dynamic nature of the peptide-protein interface, peptide resonance could not be assigned unequivocally using the traditional double-filtered experiments. This necessitated the production of uniformly ^{15}N/^{13}C-labeled peptide by recombinant approaches. To that end, a DNA fragment coding for the peptide sequence was synthesized and cloned into a pAED4 vector. This allowed the peptide to be expressed in *E. coli* as a His-tagged fusion protein in inclusion bodies. Purification of the fusion protein was accomplished using Ni-NTA agarose (Qiagen) under denaturing conditions following the manufacture's recommendations. The labeled peptide was obtained upon CNBr cleavage of the fusion protein, purified on HPLC, and identified by mass spectrometry.

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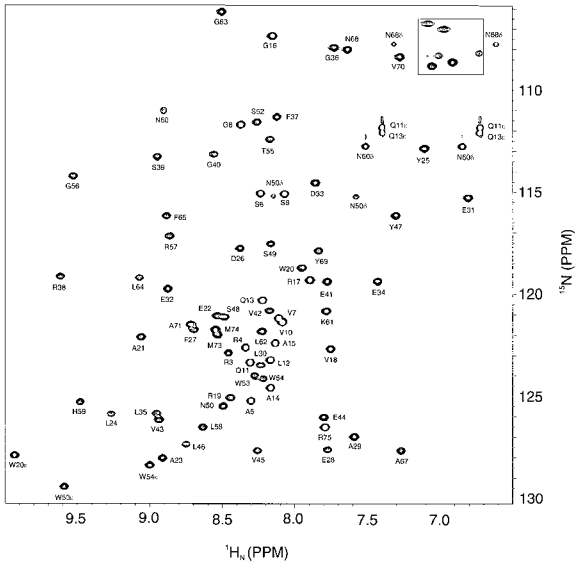

Figure 1. ^{1}H-^{15}N HSQC spectrum of Gads C-terminal SH3 domain in complex with a peptide derived from SLP-76 recorded at 12 °C. A complete assignment of the backbone amide resonance, along with those of side chains of Asn and Gln, are shown. Peaks in the boxed insert at the up-right corner correspond to the side chain H-N of Arg residues folded along the ^{15}N dimension.

Two sets of samples were prepared. For assignment of protein resonance, double-labeled SH3 protein (∼ 1.50 mM) was complexed at 1:1 ratio with unlabeled peptide. For assignment of peptide peaks, natural abundance SH3 protein was titrated into a solution containing ∼1.50 mM ^{15}N/^{13}C-labeled peptide to form a 1:1 protein-peptide complex. The complexes were formed at pH > 8.0 and buffer-changed to a medium containing 50 mM sodium phosphate (pH 6.0) and 150 mM NaCl in 90% H_2O/10% D_2O before recording NMR spectra.

All NMR experiments were performed at 12 °C on a Varian Inova 600 NMR spectrometer equipped with triple-resonance probes. The following spectra were recorded: 2D ^{1}H-^{15}N HSQC, 3D ^{15}N/^{13}C-edited NOESY-HSQC, half-filtered ^{15}N/^{13}C-edited NOESY-HSQC, HNCACB, CBCA(CO)NH, HCC(CO)NH, CC(CO)NH (for reviews, see Bax et al., 1994), 2D (Hβ)Cβ(CγCδ)Hδ, (Hβ)Cβ(CγCδCε)Hε (Yamazaki et al., 1993). All NMR data were processed using NMRPipe (Delaglio et al., 1995) and analyzed with NMRView (Johnson et al., 1994). The backbone resonance assignment was achieved mainly by a combined analysis of the HNCACB and CBCA(CO)NH spectra. The sidechain resonance were assigned primarily by analysis of an HCCH-TOCSY spectrum, and subsequently verified using HCC(CO)NH and CC(CO)NH spectra. Assignments of aromatic resonance were obtained from analyzing 2D (Hβ)Cβ(CγCδ)Hδ, (Hβ)Cβ(CγCδCε)Hε, homonuclear TOCSY, ^{1}H-^{13}C HSQC and 3D ^{15}N/^{13}C-edited NOESY-HSQC data. Based on analysis of NOE and chemical shift indices, the Gads C-terminal SH3 domain was predicted to contain all the secondary structure elements found in a typical SH3 domain (Wishart and Sykes, 1994).

Extent of assignments and data deposition

Sequence-specific resonance assignments of ^{1}H, ^{15}N and ^{13}C have been obtained for all residues of the SH3 domain except two at the extreme N-terminus of the protein which are originated from cloning artifacts. Figure 1 shows the ^{1}H, ^{15}N-correlation (HSQC) spectrum of the protein with complete amide resonance assignments indicated. Sequence-specific resonance of ^{1}H, ^{15}N and ^{13}C for the peptide were also assigned. These assignments have been deposited in the BioMagResBank database and can be accessed under the code 5388.

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