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Letter to the Editor: Assignment of backbone ^{1}H, ^{13}C, and ^{15}N resonances of the SH2 domain of human Grb14

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Abstract

The ^{1}H, ^{13}C, and ^{15}N backbone resonance assignments have been made for the Src homology 2 (SH2) domain of the human molecular adapter protein Grb14. The assignments, along with the majority of the non-aromatic side-chain ^{1}H and ^{13}C resonances are reported. The SH2 domain has been complexed with a phosphotyrosine-containing peptide (pY766) corresponding to the putative binding site in the fibroblast growth factor receptor (FGFR1). Chemical shift changes upon binding are also reported.

Biological context

The growth factor receptor bound 7 (Grb 7) family of signaling proteins has been identified based upon unique domain architecture (Ooi et al., 1995). This family is comprised of Grb 7, 10 and 14. Grb14 expression levels have been strongly correlated with breast cancer cell lines with estrogen receptor positivity; and Grb14 has been found to be highly expressed in a number of prostate cancer cell lines (Daly et al., 1996). The SH2 domain of Grb14 has been shown to bind to the Tek/Tie2 RTK (Jones et al., 1999) *in vivo*, the platelet derived growth factor receptor (PDGFR) *in vitro*, and the fibroblast growth factor receptor (FGFR1) both *in vitro* and *in vivo* (Reilly et al., 2000). While the Grb14-SH2 domain shares sequence identity of ~70% with Grb7 and Grb10, analysis of the specificity of each domain has revealed different binding preferences (Janes et al., 1997). The SH2 domains of this family are characterized by an insertion of 4–5 amino acids in the EF loop relative to all other SH2 domains described. The assignment of the resonances of the Grb14-SH2 domain yields information that can be used to characterize the specificity of this domain for its ligands, and further elucidate the role of the EF loop insertion on ligand specificity.

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Methods and results

The Grb14 protein SH2 domain was expressed in *Escherichia coli* BL-21(DE3) p-LysS cells transformed with the pGEX-2T plasmid containing the Grb14-SH2 insert (residues 426–540 of full length Grb14). This construct expresses the protein as a fusion with the glutathione-S-transferase (GST) protein to facilitate purification. The cells were grown and the protein expressed at 37 °C. The fusion protein was purified by a batch GST-affinity chromatography step, (Amersham Pharmacia, manufacturer's batch protocol) and the Grb14-SH2 domain was cleaved using a thrombin cleavage site. Para-aminobenzoic acid batch chromatography was employed to remove the thrombin, and the protein was dialyzed and concentrated. Final solution conditions for NMR experiments were 0.6–0.8 mM Grb14-SH2 domain, 50 mM sodium acetate, 100 mM NaCl, 5 mM DTT, 1 mM EDTA, 90%/10% H_2O/D_2O, and 1.5 mM sodium azide at a pH of 5.5. For the complexed FGFR1-phosphotyrosine peptide experiments, molar ratios of pY766 to Grb14-SH2 domain of 0.25:1, 0.5:1, 0.75:1, 1:1, and 1.25:1 were titrated into the 0.6 mM Grb14-SH2 protein solution.

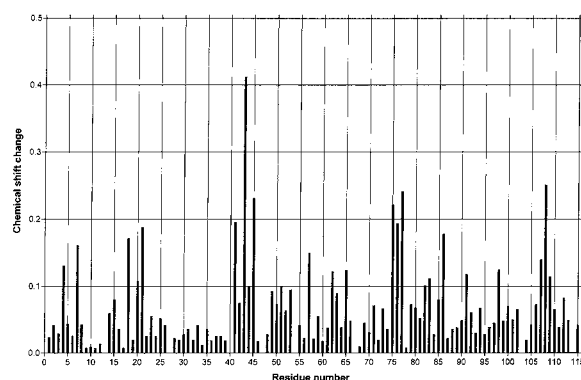

Figure 1. Above are shown the chemical shift changes (absolute values in ppm) observed in the ^{15}N HSQC spectrum of Grb14-SH2 domain upon binding FGFR1 peptide pY766. The equation $|\Delta(^{1}\text{H})| + 0.2 * |\Delta(^{15}\text{N})|$ was used, based upon previous precedent in the literature (Kim et al., 2001), to calculate the magnitude of the chemical shift change.

All NMR experiments were performed using a Varian INOVA 500 MHz NMR spectrometer. Quadrature detection in the phase-sensitive mode was performed using the method of States-TPPI (Marion et al., 1989). Processing in all dimensions sequentially involved zero-filling to the next power of two followed by multiplication with a phase-shifted sine bell curve (De Marco and Wüthrich, 1976). The amide nitrogen and hydrogen, alpha carbon, and beta carbon backbone assignments were made using data derived from the suite of 3D triple resonance experiments provided by the Varian Protein Pack including the 3D HNCA, HNCOCA, CBCACONH, CBCANH, and CCONH experiments (Kay, 1995). Side-chain and further sequential assignments were made using 3D NHSQC-TOCSY and NHSQC-NOESY experiments.

The FGFR1 peptide-bound and free Grb14-SH2 domain ^{1}H amide and ^{15}N amide resonances were identified using 2D NHSQC spectra correlated to 3D NHSQC-TOCSY spectra for spin-system identification. Figure 1 demonstrates the chemical shift changes observed in the ^{15}N HSQC spectrum upon binding the peptide in excess (1.25:1) amounts. At sub-stoichiometric amounts of the FGFR1 peptide, separate resonances for the free versus bound form of the SH2 domain were observed for the vast majority of amide resonances. For a small minority of amide resonances (approximately 5%) broad complex peaks were observed at sub-stoichiometric amounts of pY766. These results are indicative that complex formation between the Grb14-SH2 domain and the phosphorylated peptide ligand occurs in the slow to intermediate exchange time regime. At equi-molar or greater amounts of the pY766 peptide the amide resonances of the Grb14-SH2 domain remain singular and do not undergo further chemical shift change. Based upon the concentrations of pY766 and Grb14-SH2 domain used the K_d for complex formation must be less than or equal to 150 μM.

The pY766 peptide shares sequence identity to the Grb14 binding site on the human FGFR1 extracellular domain (residues 761–771). Tyrosine 766 of the FGFR1 has been shown through mutagenesis studies to interact with the Grb14-SH2 domain (Reilly et al., 2000). Compared to previous SH2 domain-phosphorylated peptide binding studies, the expected chemical shift changes in the αA, βB, BC loop, βC and βD structural components corresponding to reported observed shifts for phosphotyrosine coordination are seen. Regions in the extended EF loop which have no precedent also show significant shifts, suggesting a potential role for this insertion as an SH2 domain specificity determinant.

Extent of assignments and data deposition

All amide ^{1}H, amide ^{15}N and ^{13}C alpha and beta resonances have been assigned with the single exception of the N-terminal glycine amide nitrogen and hydrogens. Approximately 90% of the non-aromatic side chain protons and 50% of the non-aromatic Cγ and farther carbon resonances have been assigned. The listed ^{1}H, ^{15}N and ^{13}C chemical shifts for the SH2 domain of human Grb14 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5314.

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