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Letter to the Editor: Assignment of backbone ^{1}H, ^{13}C, and ^{15}N resonances of human Grb7-SH2 domain in complex with a phosphorylated peptide ligand

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Biological context

Src homology 2 (SH2) domains are conserved non-catalytic regions of approximately 100 amino acids found in a variety of cytoplasmic signaling proteins. They bind to specific phospho-tyrosine-containing sequences within autophosphorylated receptor tyrosine kinases (RTKs) and intracellular phosphoproteins, and mediate inter- and intramolecular interactions involved in signal transduction from activated RTKs (Cohen, 1995; Pawson, 1995). Growth factor receptor bound protein-7 (Grb7) is an SH2 domain-containing signaling protein encoded by a gene commonly co-amplified with the epidermal growth factor receptor-2 (EGFR2, erbB2) gene in human breast cancer cell lines and primary breast cancers (Stein, 1994). ErbB2 is over-expressed on the tumor cells of approximately 30% of breast cancer patients (Seshadri, 1993). These patients are estrogen receptor negative/erbB2 positive and are statistically disfavored for long term survival. Grb7 associates strongly with erbB2 via its SH2 domain in co-immunoprecipitation experiments (Stein, 1994). The simultaneous over-expression of Grb7 with erbB2 in breast cancer cells therefore suggests greatly amplified signaling through these proteins. Although the precise function of Grb7 is not known, it is believed to be an adapter protein linking tyrosine phosphorylated proteins to downstream effectors. Grb7, 10, and 14 together comprise the Grb7 protein family. The members of this family all contain an N-terminal Pro rich region thought to bind SH3 domain containing proteins, a central GM (Grbs and Mig) domain, and a C-terminal SH2 domain. The SH2 domains of Grb7, 10, and 14 share 68–73% residue identity. Despite this similarity, Grb10 and 14 interact only weakly with erbB2, while Grb7 binds *in vivo* to this RTK (Janes, 1997). Sequence specific assignments of the hGrb7-SH2 domain with and without bound phosphorylated peptide ligand provides the basis for investigations on the structural requirements for ligand specificity in this family of proteins.

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Methods and results

For protein expression, *Escherichia coli* BL21(DE3)-pLysS, transformed with the hGrb7-SH2-pGEX-2T expression plasmid, were grown at 37 °C. The SH2 domain (residues 435-536 of the hGrb7 protein) was expressed as a fusion protein with Glutathione S-Transferase (GST), and purified by standard protocols (Guan, 1991). Purification of the protein was achieved by binding to Glutathione Sepharose beads. The SH2 domain is released from the immobilized GST protein through the utilization of a thrombin cleavage site. Yields of pure hGrb7-SH2 were 5–6 mg per liter of culture in minimal medium. NMR samples of uniformly ^{13}C/^{15}N labeled hGrb7-SH2 domain were 0.8 mM in acetate solution (100 mM NaCl, 50 mM HOAc, 5 mM DTT, 1 mM EDTA, 0.9 mM NaN_3, and 90% H_2O/10% D_2O) at pH 6.6 and 298 K.

All NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer equipped with a ^{1}H/^{13}C/^{15}N probe and Z-axis pulsed field gradient capabilities. Quadrature detection in the phase sensitive mode was achieved by the method of States-TPPI

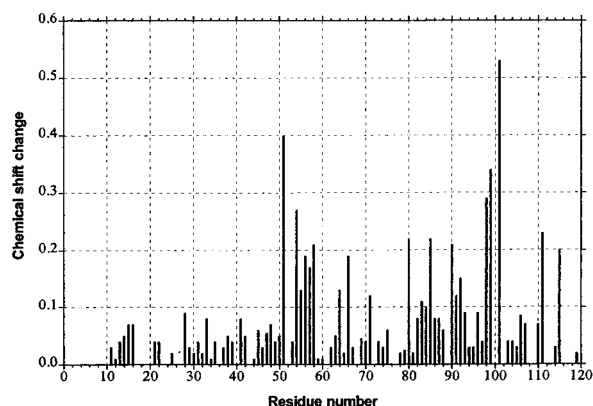

Figure 1. Above are shown the chemical shift changes (absolute values in ppm) observed in the ^{15}N HSQC spectrum of hGrb7-SH2 upon binding pY1139 (erbB2) peptide. The ratio of the two components is 1:1. The overall chemical shift change is calculated using the following equation: Chemical Shift Change = $|\Delta\delta\,(^1\mathrm{H})| + [0.2 \times |\Delta\delta\,(^{15}\mathrm{N})|]$.

(Marion, 1989). Standard processing in all dimensions included zero filling to the next power of 2 followed by multiplication by a phase shifted sine bell curve.

The assignment of the polypeptide backbone was achieved using 3D triple resonance experiments contained within the Varian Protein Pack suite (Kay, 1995). All analysis of data for sequential assignments was performed using ANSIG for Windows (Kraulis, 1994; Helgstrand, 2000). A combination of 3D HNCA, 3D HN(CO)CA, 3D HNCACB, and 3D CBCA(CO)NH experiments provided the amide proton, amide nitrogen, and alpha and beta carbon resonances for each residue.

Correlation of the ^{1}H amide and ^{15}N amide hGrb7-SH2 resonances in the liganded versus the un-liganded state were provided by spin system identification in the 3D HNCACB and 3D CBCA(CO)NH experiments of the SH2 domain both free and in complex with peptide. The 10 amino acid peptide (pY1139) ligand is identical in sequence to the Grb7 binding site on the erbB2 intracellular domain. Figure 1 provides a visual characterization of the extent of chemical shift changes seen in the ^{15}N HSQC spectrum of the hGrb7SH2 domain upon binding phosphorylated peptide ligand. Based upon homology with the Src SH2 domain (Janes 1997) areas of greatest chemical shift change upon ligand binding correspond to the βC-beta strand, βD-beta strand, and EF loop (the loop connecting βE- and βF-beta strands) regions.

Extent of assignments and data deposition

All amide ^{1}H, amide ^{15}N, and ^{13}C alpha and beta resonances were assigned for the 120 amino acid hGrb7-SH2 domain/pY1139 complex with the exception of the N-terminal Glycine and Serine residues. These residues were either non-observable in all spectra, or observable only in the ^{15}N nOe (3 s mixing time) nitrogen-proton correlation spectrum.

The amide ^{1}H, amide ^{15}N, and alpha and beta ^{13}C resonances for hGrb7-SH2/pY1139 domain complex at pH 6.6 and 298 K have been deposited in BioMagResBank, accession number BMRB-5327 (http://www.bmrb.wisc.edu).

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