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Main-chain signal assignment for the PDZ2 domain from human protein tyrosine phosphatase hPTP1E and its complex with a C-terminal peptide from the Fas receptor*

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Biological context

PDZ domains belong to a group of modules mediating interactions between proteins. Most proteins containing PDZ domains are associated with receptors, membrane channels and signal transduction proteins (for reviews, see Ponting et al., 1997; Saras and Heldin, 1996). It has been shown that a consensus sequence of the last three amino acids, T(S)-x-V, is critical for binding to PDZ domains. We are studying the structure of the second PDZ domain from the human cytosolic phosphatase hPTP1E (Banville et al., 1994). This domain (PDZ2) mediates interactions between hPTP1E and the Fas receptor and was shown to act as a negative regulator of Fas-mediated apoptosis (Sato et al., 1995). The C-terminal fifteen-amino-acid fragment of Fas matches the PDZ binding consensus sequence and is critical for Fas-hPTP1E interaction (Sato et al., 1995). In this work we present backbone assignments for the PDZ2 domain from hPTP1E (amino acids 1361–1456 in the phosphatase sequence), and its complex with a 15-amino acid fragment from the Fas receptor with the sequence D-S-E-N-S-N-F-R-N-E-I-Q-S-L-V.

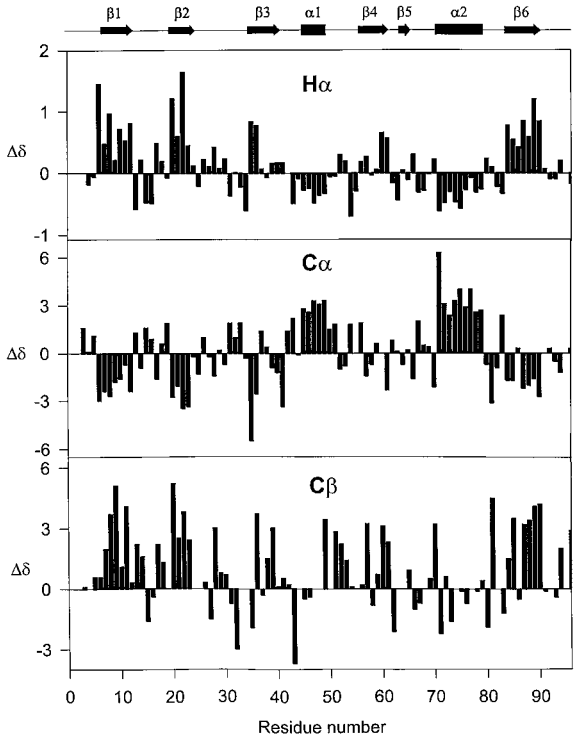

Figure 1. Deviations from random coil chemical shifts of H^{α}, C^{α}, and C^{β} resonances (in ppm) in PDZ2 as a function of residue number. Random coil chemical shift values are those of Wishart and Sykes (1994). Secondary structure elements according to the X-ray structure of the third PDZ domain from the brain synaptic protein PSD-95 (Doyle et al., 1996) are shown schematicallyat the top.

*These data have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRM accession numbers 4123 and 4124.

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Methods and results

^{15}N-enriched and ^{15}N/^{13}C-enriched PDZ2 domain (1361-1456) from cytosolic phosphatase hPTP1E was produced by growth of *E. coli* on a minimal medium containing ^{15}N-ammonium sulfate and ^{13}C-glucose as sources of nitrogen and carbon, respectively. Protein purification was performed using ion exchange, followed by size exclusion chromatography (Ekiel et al., in preparation). PDZ2 samples for NMR experiments were 3.0–5.0 mM in 50 mM sodium phosphate buffer, 0.15 M NaCl at pH 6.9 at 293 K. All 3D triple resonance NMR experiments were performed using a Bruker DRX500 spectrometer equipped with an actively shielded x, y, z gradient triple resonance probe and Bruker pulse field gradient accessory. For the heteronuclear 3D experiments, the carrier frequency was shifted to the middle of the NH frequencies (7.3 ppm), to increase digital resolution in the proton dimension. DIPSI-2, WURST-20 and WALTZ-16 sequences were used for heteronuclear decoupling in proton, carbon and nitrogen dimensions, respectively. Water suppression was achieved either by presaturation or the WATERGATE sequence (Piotto et al., 1992).

Complete data for the sequential assignment of the backbone signals were collected using ^{15}N/^{13}C-enriched samples and 3D HNCA, HNCO, HN(CO)CA, CBCA(CO)NH, HNCACB, HNCAHA, HCCH-COSY, HCCH-TOCSY, NOESY-HMQC and TOCSY-HMQC experiments (for a review, see Bax and Grzesiek, 1993). Additional HNCO, HN(CA)HA and HBHA(CO)NH experiments were used to resolve problems of signal overlap and carbon chemical shift ambiguities. The assignment of Lys-2, which is located between two prolines, was achieved using a proline-edited HACA(CO)(N) experiment. For protein resonance assignment in the PDZ2-peptide complex, a 3D NOESY-HMQC experiment was performed and results compared with those for the free PDZ2 domain. Most amides show similar NOE patterns in the free PDZ2 and complex spectra. This allowed the NOESY-HMQC spectra to be used directly for signal assignment. Additionally, the similarity of NOEs (both presence and intensities of cross-peaks) suggested a very similar overall fold in the protein in both ligand free and liganded forms. NMR spectra were processed using GIFA (Delsuc, 1989) and XWINNMR software on Silicon Graphics computers. All 2D and 3D datasets were linear-predicted and/or zero-filled once and a phase-shifted sine bell squared window function was applied prior to Fourier transformation.

The secondary structure was analyzed using proton and carbon chemical shifts (Figure 1), applying the methodology developed by Wishart and Sykes (1994). C^{β}, C^{α} and H^{α} chemical shifts confirmed the location of the α-helix, and β-sheet strands characteristic for the PDZ fold (Doyle et al., 1996). The HSQC spectra of the PDZ2/peptide complex showed intermediate exchange rates, and significant changes of the chemical shifts upon complex formation were observed in extensive regions of the protein (to be discussed elsewhere).

Extent of assignments and data deposition

Sequence specific assignments ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{1}H^{\alpha}$, $^{13}C^{\beta}$, $^{1}H^{\beta}$) were obtained for all amino acids in the PDZ2 domain with the exception of the amide signals of H32, which could only be detected at low (<5) pH values, and prolines P1 and P95. The signals of L18, H32 and R79 were not assigned in the complex. Chemical shifts were measured relative to internal DSS for ^{1}H and calculated assuming $\gamma_N/\gamma_H = 0.101329118$ and $\gamma_C/\gamma_H = 0.251449530$ (Wishart et al., 1995). The ^{1}H, ^{13}C and ^{15}N chemical shifts for the PDZ2 domain have been deposited in BioMagRes Bank (http://www.bmrb.wisc.edu) under accession number 4123, and amide signals for the complex under accession number 4124.

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