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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of the targeting (FAT) domain of focal adhesion kinase

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Biological context

Focal adhesion kinase (FAK) is a nonreceptor kinase that can be activated by integrin signaling. Since the discovery of FAK in the early 1990s, this protein and its related signaling pathways have been studied in some detail (Cary and Guan, 1999; Schlaepfer et al., 1999). It is now clear that FAK plays an important role in relaying the signals that are generated by the attachment of cells to the extracell matrix (ECM), and which are transmitted through integrins to cytoplasmic and nuclear targets. In this way, FAK regulates cellular processes such as migration, survival, and proliferation (Parsons et al., 2000). The C-terminal part of FAK is rich with protein-protein interaction sites. In certain cells, this part of FAK is autonomously expressed (Hildebrand et al., 1993) and termed FAK-related non kinase (FRNK). FRNK contains a C-terminal focal adhesion targeting (FAT) domain and several proline-rich regions that serve as docking sites for many SH3-containing proteins. It acts as an endogenous inhibitor of FAK signals (Taylor et al., 2001). Upon activation, FAK colocalizes at focal adhesions (Cary and Guan, 1999; Schlaepfer et al., 1999), and mutation studies have shown that the FAT domain is responsible for this localization.

Much is known about the role of FAK in integrin signaling. However, the mechanism by which activated FAK localizes to focal adhesions remains unclear. Although extensive genetic and biochemical studies of this area have been performed (Schlaepfer et al., 1999), the lack of detailed structural information about FAK and the proteins with which it interacts has prevented investigators from fully understanding the results of such studies. To help understand the mechanisms of focal adhesion targeting of the FAT domain, we have started the NMR structure determinations of the FAT domain, and the interactions between the FAT domain and one of its binding partners, paxillin. Here we report complete sequence-specific assignments for the FAT domain in the context of the complex formed by the FAT domain bound to a peptide corresponding to the LD2 sequence of paxillin.

Methods and experiments

The chicken FRNK cDNA was kindly provided by Dr J. Thomas Parsons (University of Virginia). The cDNA encoding the FAT domain (residues 916-1053) was subcloned into PET28a vector. The N-terminal His-tagged FAT domains were subsequently expressed in *E. coli*. The methods of protein induction, harvest, and purification have been described previously (Wong et al., 2000). To isotope-label protein, we used MOPS-buffered medium that contained ^{15}NH4Cl (1 g l^{-1}) and $^{13}C_6$-glucose (2.5 g l^{-1}). The LD2 peptide of chicken paxillin (residues 139 to 162) was chemically synthesized. The samples of the FAT domain:LD2 peptide complex were generated by titrating increasing amounts of unlabeled LD2 peptide with ^{15}N-labeled or ^{15}N, ^{13}C-labeled FAT domain. The progress of the titration was monitored by recording 2D ^{1}H-^{15}N correlated spectra. Sample concentrations for NMR experiments were typically 0.5 to 1.6 mM in 10 mM potassium phosphate buffer (pH 6.5) and 0.1% NaN_3. The molar ratio between the FAT domain and the LD2 peptide in the NMR samples was 1:10

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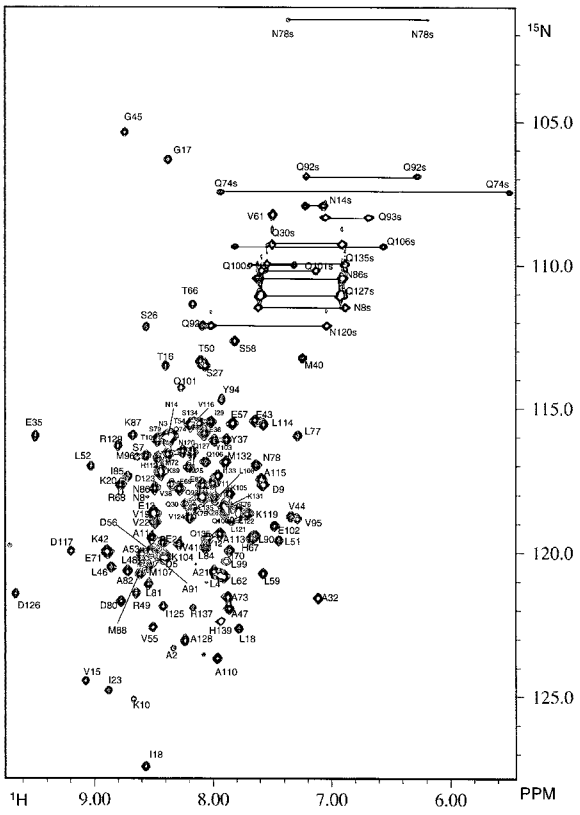

Figure 1. ^{1}H, ^{15}N HSQC spectrum of uniformly ^{15}N-enriched 1 mM FAT (FAK916-1053) in 90% H_2O/10% D_2O recorded at pH 6.5 and 310 K. The one-letter amino acid code and the sequence number indicate backbone resonance assignments, residues 2-139 reported here correspond to the residues 916-1053 of FAK.

All NMR data were acquired with Varian Inova 600-MHz spectrometers at 37 °C. Proton chemical shifts are referenced to H_2O peaks (4.75 ppm), ^{13}C and ^{15}N chemical shifts are referenced indirectly to DSS, using the absolute frequency ratios. Data were processed and displayed by the program packages NMRpipe and NMRDraw (Delaglio et al., 1995) on an SGI Octane workstation. The program XEASY (Xia et al., 1993) was used for data analysis and assignment. For the LD2-bound FAT domain, backbone resonances were assigned on the basis of 3D HNCA, HNCACB, CBCA(CO)NH, HNCO, and HNCOCA, whereas aliphatic side-chain resonances were assigned on the basis of 3D ^{15}N-edited TOCSY, HCCH-COSY, and HCCH-TOCSY spectra. Aromatic side-chain resonances were assigned on the basis of 2D NOESY and TOCSY of the D_2O sample. Residual gaps and ambiguities were resolved by using sequential NOEs measured in 3D ^{15}N-edited NOESY and ^{13}C-edited NOESY, with the help of 4D $^{15}N/^{13}C$ NOESY and 4D $^{13}C/^{13}C$ HMQC-NOESY-HSQC.

Extent of assignments and data deposition

The backbone resonances assignments were essentially complete for all residues observed in the ^{1}H-^{15}N HSQC spectrum (Figure 1). Residues 2-139 of FAT reported here correspond to the residues 916-1053 of FAK. Residues with missing assignments are ASN3, ARG6, SER65 and SER98. The amide protons of these unassigned residues were not observed, presumably due to the fast exchange with the water. In total, 96% of $^{13}C_{\alpha}$, $^{1}H_{\alpha}$ and $^{13}C_{\beta}$, 90% of $^{13}C_{O}$, 96% of $^{1}H_{\beta}$ and more than 95% of other side-chain resonances (^{1}H and ^{13}C) were determined from 3D and 4D triple resonance experiments. Chemical shift assignments have been deposited in the BioMagResBank database (accession number 5266).

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