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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of the death domain of TRADD

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Biological significance

The tumor necrosis factor (TNF) and tumor necrosis factor receptor (TNFR-1) are representative members of a superfamily of proteins that play critical roles in signaling pathways (Locksley, 2001). Cytoplasmic signaling downstream of TNFR leading to cellular responses is mediated by TNF receptor-associated factors (TRAFs) and 'death domain' (DD) containing adaptor proteins (Arch et al., 1998). The DD of TNFR-1 recruits the DD of TRADD (TNF receptor-associated death domain), which in turn recruits other DDs (Hsu et al., 1995, 1996a, b). These protein-protein interactions, which are based on homo- and hetero-dimerization, form part of the TNFR-1 signaling complex, leading to the induction of apoptosis and activation of two major transcription factors, NF-kB and c-Jun which are responsible for cell growth, development as well as immune, inflammatory and stress responses.

The DD is a ∼90 amino acid domain that functions as an adaptor domain involved in homotypic interactions and is found in multiple death receptor pathways (Aravind et al., 1999). Although DD modules share overall structural homology, based on the structure determination of several DD to date, the structural details of distinct DD and DD-DD interactions likely hold the key to the high degree of signaling specificity mediated by DD proteins. In an effort to better understand specificity of signaling mediated by TRADD, which includes self-association and binding to DDs of TNFR1, Rip and FADD, we have initiated structural studies of the DD of TRADD, which is included in residues 196-301 of the full-length TRADD protein. We report near complete ^{1}H, ^{13}C, and ^{15}N assignments for the TRADD-DD. We anticipate that the structure determination of the TRADD-DD will allow further comparative analysis of the DD class of signaling modules and will illuminate the details of TRADD mediated signaling pathways downstream of TNFR-1.

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Methods and experiments

The C-terminal hits-tagged TRADD-DD pRSFTb was expressed in BL12 (DE3) E.coli, with $^{15}NH_4SO_4$ (2 g/L) and $^{13}C_6$-glucose (2 g/L). Cell pellets were resuspended in 50 mM Tris pH 7.5, 50 mM NaCl, and a protease inhibitor tablet 'Complete' (Boehringer Mannheim) was added per 50 ml. The suspension was disrupted by passing 5 times through a microfluidizer (Microfluidics Corp, Newton, MA). After centrifugation, the pellet was extracted in 50 mM Sodium Acetate pH 4.2, 50 mM Magnesium Sulfate and 5 mM DTT. The combined extractions were dialyzed against 10 mM Sodium Acetate pH 4.2, 5 mM DTT overnight at 4 °C. After concentration with a Millipore Ultrafree filtration device to around 1 mg/ml, the sample was loaded to a TSK gel G2000sw column (Tosoh Bioseph LLC). Fractions containing TRADD-DD were pooled and concentrated. NMR samples contained 0.6–1.1 mM TRADD-DD in 10 mM NaOAc, pH = 4.2, 5 mM DTT in 100% D2O or 95% H2O/5%D2O. All NMR experiments were collected at 30 °C on a Bruker Avance 600 spectrometer. Backbone and side chain assignments were obtained from the following experiments: 2D ^{1}H-^{15}N HSQC,

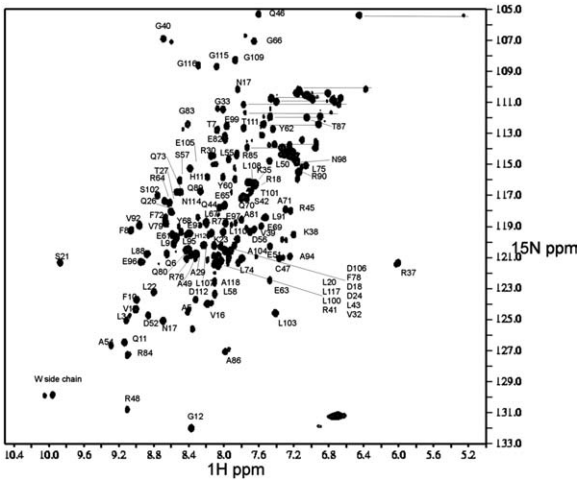

Figure 1. ^{1}H-^{15}N HSQC spectrum of TRADD-DD acquired at 600 MHz, pH = 4.2, 30 °C. Assignments of the backbone are shown by the single-letter code and residue number. Minor peaks are not marked, and are due mostly to residues near *cis-trans* proline isomers.

2D ^{1}H-^{13}C HSQC, HNCACB, CBCA(CO)NH, ^{15}N-NOESY-HSQC, CC(CO)NH-TOCSY, HCC(CO)NH-TOCSY. Spectra were processed with NMRPipe software (Delaglio et al., 1995) and analysed with PIPP (Garrett et al., 1991).

Extent of assignments and data deposition

Figure 1 shows the ^{1}H-^{15}N HSQC of TRADD-DD. The amino acid numbering used here starts withM1, P2, P3 and ends with A118, which corresponds to P196 to A312 in the native TRADD protein. The HN, ^{15}N, ^{13}Cα, ^{13}Cβ and side chain ^{13}C and ^{1}H assignments are nearly complete. Resonances for residues M1, P2, P3 and the last two histidines of the C-terminal his-tag were not assigned. The backbone and side chain assignments of TRADD-DD have been deposited in the BioMagResBank database (http://www.bmrb.wis.edu) under accession number 5862.

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