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Letter to the Editor: NMR assignment of the *Drosophila* Argonaute2 PAZ domain

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Biological context

The PAZ domain (named after the three proteins Piwi, Argonaute and Zwille) is found exclusively in two protein families, the Argonaute and Dicer families (Cerutti et al., 2000). Members of both families were shown to be necessary for a conserved biological response to double-stranded RNA (dsRNA) called RNA interference (RNAi) or Posttranscriptional Gene Silencing (PTGS) (Denli and Hannon, 2003). In the process of RNAi, the dsRNA leads to the degradation of the cognate mRNA in two steps. In the initiation step, dsRNA is cleaved by the RNase III-like enzyme Dicer into 21–23-nucleotide small interfering RNAs (siRNAs). In the effector step, the oligomeric RNA-induced silencing complex (RISC) targets messenger RNAs homologous to the siRNAs for degradation. The Argonaute2 protein (Ago2) was identified to be a critical component of the RISC (Hammond et al., 2001). We have determined the three-dimensional structure of the PAZ domain of *Drosophila melanogaster* Ago2 by NMR spectroscopy in order to gain insight into its function (Lingel et al., 2003). Here, we report complete sequence-specific assignments for the Ago2 PAZ domain.

Methods and experiments

The *Drosophila melanogaster* Ago2 PAZ domain (TrEMBL entry Q9VUQ5; Met 605 to Ser 743) was amplified from a S2 cell cDNA library and cloned via *Nco*I and *Bam*HI restriction sites into the pETM60 vector, derived from pET24-d (Novagen) (see http://www.embl-heidelberg.de/ExternalInfo/geerlof/draft_frames/index.html). The fusion protein was expressed at room temperature in the *E. coli* strain BL21(DE3). For labeling of the protein uniformly with $^{15}N/^{13}C$ or ^{15}N, cells were grown in M9 minimal medium supplemented with $^{15}NH_4Cl$ with or without $^{13}C_6$-glucose. Cell lysates were subjected to affinity chromatography purification using Ni-NTA Superflow material (Qiagen), followed by cleavage of the tag with TEV-protease overnight. After a second affinity purification step, the protein was purified to homogeneity by gel filtration. The purified protein consisted of the cloned 139 residues from Ago2 preceded by four residues (GAMA) from the TEV-cleavage site and the restriction site. NMR samples at 1.0–1.5 mM concentration were prepared in 50 mM sodium phosphate buffer (pH 6.8), 150 mM NaCl, 0.2 mM DTT in H_2O or ^2H_2O.

NMR spectra were acquired at 22 °C on Bruker DRX500, DRX600, DRX700 or DRX900 spectrometers equipped with triple resonance or cryogenic probes (500 and 600 MHz). Spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed using NMRVIEW (Johnson and Blevins, 1994). The ^1H, ^{13}C, and ^{15}N chemical shifts were assigned by standard methods (Sattler et al., 1999). Chemical shift assignments were based on HNCA, HNCOCA, CBCA(CO)NH, HNCACB, (H)CCONH-TOCSY, H(C)CONH-TOCSY, HCCH-TOCSY. NOE assignments were derived from 3D $^1H,^{15}N$-HSQC-NOESY, NOESY-$^1H,^{13}C$-HMQC and a 3D version of a $^1H,^{15}N$-HSQC-NOESY-(^1H),^{13}C HMQC experiment recorded in H_2O. $^3J(HN,H\alpha)$ couplings measured in an HNHA-J experiment (Kuboniwa et al., 1994) were used to constrain ϕ. Additional ϕ, and ψ restraints were derived from TALOS (Cornilescu et al., 1999). $^1D\ H^N$-N residual dipolar couplings were measured in liquid crystalline medium (Rückert and

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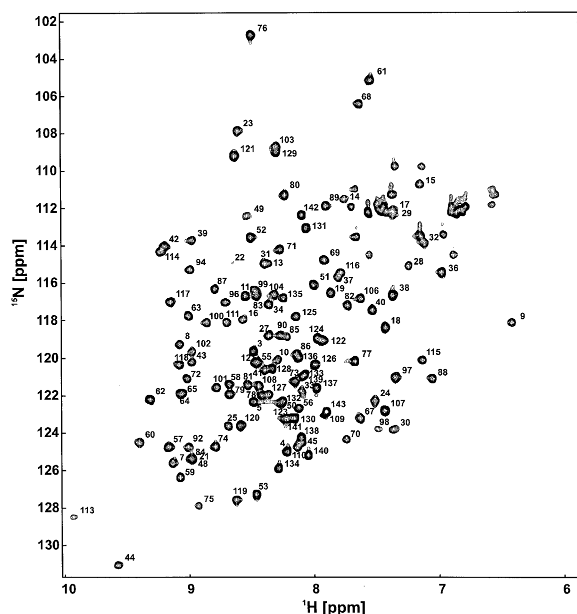

Figure 1. ^{1}H,^{15}N HSQC spectrum of a ^{15}N-labeled 1.0 mM sample of the *Drosophila* Argonaute2 PAZ domain in 95:5 H_2O:D_2O. Residues numbers are indicated (shifted by −600 compared to the full-length protein, i.e. Met 5 corresponds to Met 605 in the Argonaute2 protein).

Otting, 2000) with an axial component/rhombicity of the alignment tensor of 16.2 Hz and 0.43, respectively.

Extent of assignments and data deposition

The assignments of backbone and side chain resonances are essentially complete (H_N, N, Cα > 98%; ^{1}H and ^{13}C side chains > 95%). Only Gly 1 and the amide groups of Ala 2 and Ser 105 are missing. The side chain amides of Gln, Asn, Arg and Lys have not been assigned. The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank (*http://www.bmrb.wisc.edu/*) under the BMRB accession number 5999. The coordinates of the Ago2 PAZ domain have been deposited at the Protein Data Bank under accession number 1upo.

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