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Letter to the Editor: NMR assignment of human ASC2, a self contained protein interaction domain involved in apoptosis and inflammation

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Biological context

ASC_2 is the sole member of the pyrin family of proteins that exists as a single protein domain (Pawlowski et al., 2001). Most members of this gene family are expressed as multimodular proteins with recruitment domains involved in apoptosis and inflammation (Berlin and DiStefano, 2000). The exact role of the ASC_2 protein is unknown to date; it may modulate protein-protein interactions. Heterodimerization of ASC_2 with other members of the same gene family may inhibit or activate the function of the multidomain members. Theoretical fold prediction studies have rendered inexact secondary structure limits for the pyrin domain based on sequence homologies (Martinon et al., 2001; Fairbrother et al., 2001; Pawlowski et al., 2001). Exact boundaries are needed to design mutation studies to probe putative protein interaction activities. We report complete sequence specific backbone NMR assignments. These assignments provide the first experimentally determined $\Delta C\alpha$ patterns useful to delineate putative structural boundaries based on NMR solution studies.

Methods and experiments

Recombinant ASC_2 was expressed in *E. coli*. We obtained a plasmid containing ASC_2 fused to GST from Dr J. Reed's laboratory. The expressed protein was unstable at 30 °C due to a secondary thrombin site within the sequence. The DNA fragment was excised, purified and ligated into the vector PGEX-P and cleaved from GST with a different protease. The expressed protein was stable for at least 6 months and additional unstructured N-terminal residues from the modified cleavage site rendered ASC_2 soluble. Unlabeled, ^{15}N labeled and ^{13}C, ^{15}N labeled samples in concentrations ranging from 0.8 mM to 2 mM were produced. Typical samples had 9.8 mg ml^{-1} of dissolved protein in 550 µl of 90% H_2O pH 7.3. The buffer components were 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 140 mM NaCl and 0.02% NaN_3. The purified protein migrated as a monomer in gel filtration experiments. NMR measurements were performed at 30 °C on a 500 MHz Unity Plus Varian spectrometer equipped with pulse field gradient triple-resonance probes. Proton chemical shifts, ^{13}C and ^{15}N chemical shifts are referenced using published methods (Wishart et al., 1995). NMR spectra were processed using Felix version 2000. Sequence specific assignments were obtained using 3D HNCA, 3D HN(CO)CA, 3D CBCA(CO)NH, 3D HNCACB, 3D HNCO, 3D C(CO)NH and 3D HC(CO)NH (Grzesiek et al., 1993; Cavanagh et al., 1996). Ambiguous assignments were probed by examining ^{15}N 3D NOESY HSQC and ^{15}N 3D TOCSY HSQC, and by sequential assignments based on 2D ^1H ^1H NOESY spectra (Wütrich, 1986) obtained at 500 MHz and at 600 MHz. Identification of the amino acids started from characteristic chemical shifts of Cβs and Cαs, Hβs and Hαs determined using heteronuclear experiments obtained on the doubly labeled sample. Aromatic rings were identified from 2D ^1H-^1H NOESY data in D_2O.

Extent of assignment and data deposition

All backbone resonances (Cα, CO, N,H) were assigned with the exception of the ^{15}N values for the proline moieties. All Cβ and CO values are reported, with the exception of the Cβ and CO values of residues preceding prolines. 90% of the sidechain proton resonances (Hβ, Hα, etc.) are included. Initial sec-

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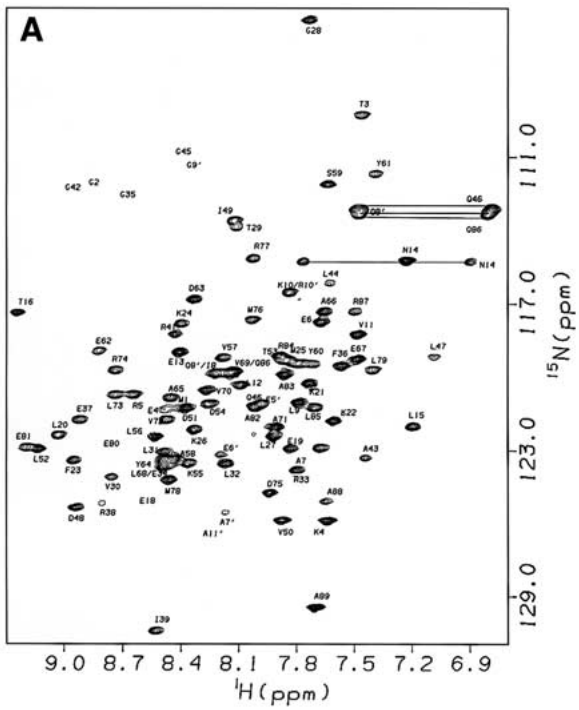

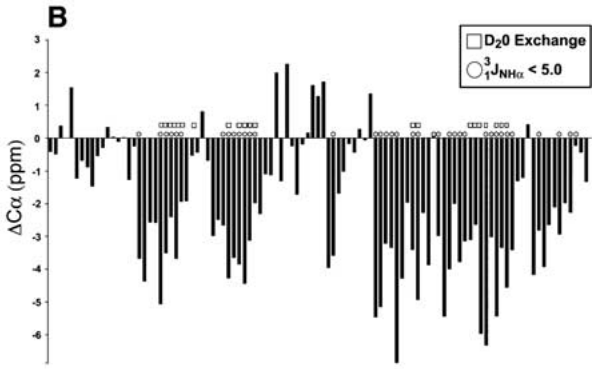

Figure 1. A 500 MHz ^{1}H ^{15}N-HSQC spectrum of recombinant ASC_2 obtained at 300 K. Resonance assignments are indicated with the one-letter amino acid code and residue number. Side-chain amide protons of Asn and Gln are indicated by horizontal lines. (B) Plots representing ΔCα values obtained from ΔCα = Cα obs − Cα random coil values (www.bmrb.wisc.edu/ref_info/csishift.txt) indicate structured segments. Data for the amide protons resistant to D_20 exchange at pH 7.3 for 3hrs assayed by HSQC every 20 min (squares) and ^{3}JNHα values below 5.0 Hz estimated from HNHA spectra (circles) are also depicted.

ondary structure identification (Figure 1B) was based on chemical shift differences between the observed Cα and the value for random coil Cα. We also obtained ΔHα and ΔCO values (not shown). Proton-D_2O exchange experiments and ^{3}JNHα values confirmed secondary structure elements. Available ^{1}H, ^{13}C and ^{15}N chemical shifts from the 103 residue construct have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5233.

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