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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C backbone assignment of MJ1267, an ATP-binding cassette

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Biological context

The ATP-binding cassette (ABC) is the signature module of the ABC transporter superfamily (Ambudkar et al., 1999). ABC transporters comprise a significant percentage of all non-viral genomes and transport a variety of important cargos, including ions, amino acids, lipids, sugars and proteins, across cell and organelle membranes. ABC transporters are associated with a diverse number of pathologies, such as multidrug resistance and cystic fibrosis (Ambudkar et al., 1999).

All ABC transporters have the same basic molecular architecture (Higgins, 1995). A pair of transmembrane domains, generally containing six transmembrane helices, bind solutes at one side of the membrane and release them at the other side. Energy for transport is obtained from ATP hydrolysis catalyzed by a pair of ABCs. These components are fused into a single polypeptide chain in most eukaryotes but are often expressed as separate polypeptides in lower organisms. Substantial sequence diversity exists in trans-membrane domains, presumably reflecting the diverse substrate specificity of ABC transporters. However, all ABCs bear strong sequence similarity, which implies a common mechanism of energy generation and transduction.

An abundance of structural information about ABCs (for a review see Kerr, 2002) and ABC transporters (Locher et al., 2002) has been obtained from X-ray crystallography in recent years. However, ABCs have been refractory to solution NMR studies that could provide complementing mechanistic information (Duffieux et al., 2000). MJ1267 is the ABC domain of a branched amino acid transporter from the hyperthermophilic archaebacterium (*Methanococcus jannaschii*). Crystal structures of the apo and ADP-bound MJ1267 have been solved (Karpowich et al., 2001). The backbone assignment of MJ1267 is the first step towards understanding the structure, dynamics, and function of ATP-binding cassettes in solution.

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Methods and experiments

[*U*-^{13}C; *U*-^{15}N; *U*-97% D] MJ1267 (residues 1–257) was overexpressed using triply labeled BIOEXPRESS medium (Cambridge Isotopes). [*U*-^{13}C;*U*-^{15}N] MJ1267 was overexpressed using doubly labeled M9 medium. Both samples were purified as previously described (Karpowich et al., 2001). A 0.6 mM [*U*-^{13}C; *U*-^{15}N; *U*-97% D] MJ1267 sample in 20 mM sodium phosphate buffer (pH 7.0 and 1 mM NaN_3) was used for TROSY versions (Salzmann et al., 1998) of HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB and HN(CO)CACB experiments for backbone assignments (Cavanagh et al., 1996). These experiments were performed on a Varian INOVA 600 MHz spectrometer at 313 K. A 0.5 mM [*U*-^{13}C;*U*-^{15}N] MJ1267 sample was used for HNCA, HN(CO)CA and HNCO experiments performed on a Bruker DRX600. Proton chemical shifts were measured relative to an external DSS standard and ^{13}C and ^{15}N chemical shifts were referenced indirectly (Cavanagh et al., 1996). NMR spectra were processed with nmrPipe

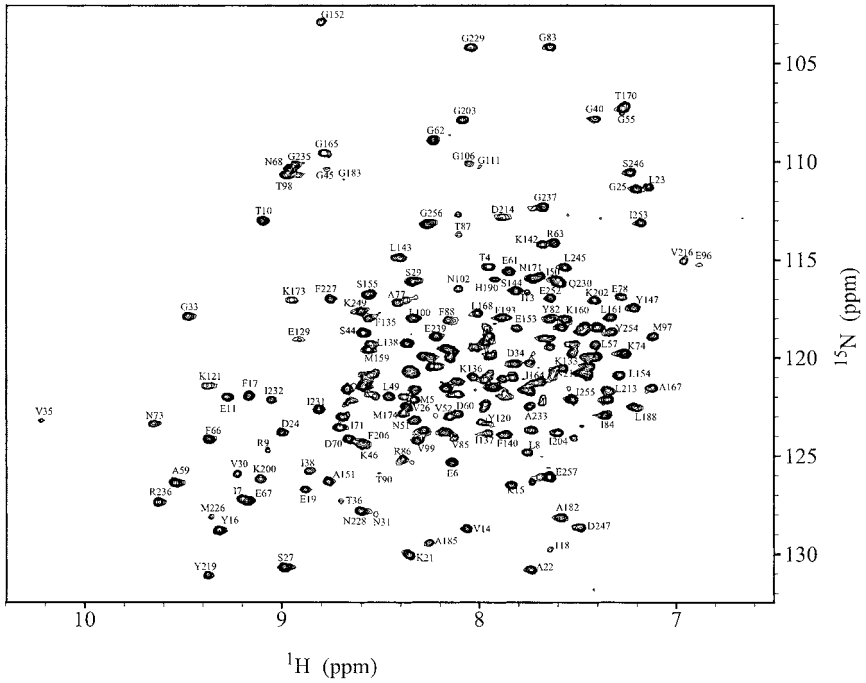

Figure 1. ^{1}H-^{15}N TROSY 2D correlation spectrum of MJ1267 at 313 K and pH 7.0, collected at 600 MHz. The assignments of the peaks are labeled with their one-letter amino acid codes and sequence number. Some labels have been omitted for clarity.

(Delaglio et al., 1995) and analysed with Sparky (T.D. Goddard and D.G. Kneller, SPARKY 3, University of California, San Francisco). The autoassign program (Moseley et al., 2001) was used to established an initial ∼70% of assignments. About 15 amide deuterons were evidently not exchanged back to protons during purification of the perdeuterated sample and the assignment of these residues were obtained from the experiments performed on the doubly labeled sample.

Extent of assignments and data deposition

91% of the non-proline backbone ^{1}H and ^{15}N resonances have been assigned. The unassigned residues are M1, R2, D3, G18, N42, G43, Q92, L104, I108, N109, S117, L118, H145, G156, G157, G187, L207, I208, I209, E210, H211, L223 and Y224. The reduced signal intensities of these residues presumably result from chemical and/or solvent exchange. The C^{α}, C^{β} and CO resonances were obtained sequentially from amide assignments. Figure 1 shows the ^{1}H-^{15}N 2D TROSY correlation spectrum. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5462.

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