Journal of Biomolecular NMR **82:** 191–192, 2004.
KLUWER/ESCOM

Letter to the Editor: Backbone resonance assignment of the L-arabinose binding protein in complex with D-Galactose

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Received 26 February 2003; Accepted 14 April 2003

Key words: carbohydrate binding protein, *E. coli*, NMR assignments

Biological context

L-Arabinose binding protein (ABP) is a member of a family of proteins found in the periplasm of Gram-negative bacteria. All these proteins serve as initial components of osmotic shock-sensitive active transport systems for a variety of carbohydrates, amino acids and ions. More importantly, ABP has proved to be an excellent, well-defined system for the detailed investigation of structure-activity relationships, in the protein-carbohydrate complexes field by X-Ray crystallography (Quiocho, 1993). An NMR assignment of this protein was undertaken as a prerequisite to study how the domains of the protein change and for characterizing the dynamical properties of the protein in solution upon ligand binding.

Methods and experiments

The *E. coli* strain overexpressing ABP was a generous gift from Prof F. Quiocho (Yale University). Recombinant, uniformly ^{15}N (>95%), ^{13}C (>95%), ^{2}H (50%) labelled ABP was over-expressed at 37 °C. Stable isotopes were incorporated using a medium consisting of 75% Celtone-dCN (Spectra Stable Isotopes) and 25% of M-9 minimal medium containing uniformly labelled ^{13}C, 50%^{2}H-glucose, ^{15}N-ammonium chloride and D_2O (50%). The cells were harvested by centrifugation after an eight hours culture and the periplasmic space content was selectively extracted using the osmotic shock procedure (Neu and Heppel, 1965). The soluble protein was purified by anion exchange (DEAE Sepharose) followed by gel-filtration chromatography (Superdex 200, Pharmacia). Approximately 20 mg of labelled ABP were obtained per litre of culture.

A 300 µl NMR sample was prepared containing approximately 1.5 mM ABP, 20 mM potassium phosphate (pH 7), 3 mM sodium azide, 0.1 mM EDTA, 50 µM protease inhibitor (Pefabloc), 10% D_2O and 3 equivalents of D-galactose. All NMR experiments were recorded at 308 K on a Varian INOVA 600 spectrometer equipped with a triple resonance z-gradient probe. Assignments were derived from standard and TROSY versions of ^{15}N-HSQC, HNCA, HN(CO)CA, HNCO, HN(CA)CO, HN(CA)CB, HN(COCA)CB and ^{15}N-NOESY-HSQC experiments (Sattler et al., 1999). NMR acquisition parameters (C means complex points) for 3D experiments were the following: The spectral windows (sw) for ^{1}H and ^{15}N were always 7000 Hz and 2500 Hz respectively. A delay of 1.2 s between every transient was used. For HNCA the number of transients (nt) used were 16 and the number of points acquired, in the ^{15}N, ^{13}C, ^{1}H order, were 48C, 34C and 1024C. For the HN(CO)CA (nt = 8, 40C, 35C, 1024C, sw Cα = 3800 Hz), HN(CA)CB (nt = 8, 38C, 128C, 1024C, sw Cβ = 8000 Hz), HN(COCA)CB (nt = 32, 32C, 38C, 1024C, sw Cβ = 6100 Hz), HNCO (nt = 8, 44C, 69C, 1024C, sw C′ = 1800 Hz), HN(CA)CO (nt = 32, 38C, 34C, 1024C, sw C′ = 2000 Hz), ^{15}N-NOESY-HSQC

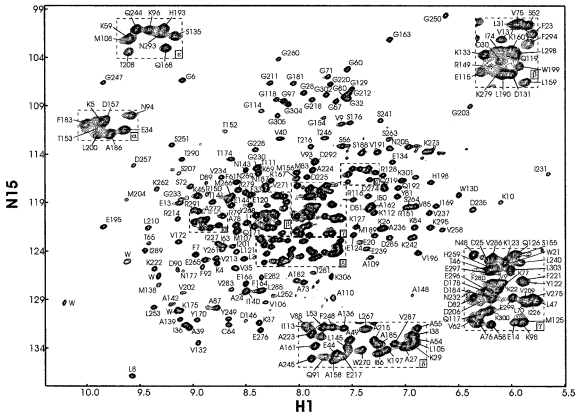

Figure 1. 2D ^{1}H-^{15}N TROSY spectrum of the *E. coli* ABP/D-galactose complex at 308 K and 600 MHz. Assignments of backbone resonances are indicated using one-letter code for amino acids and the residue number, while those tentatively assigned to Trp side-chain indole protons are designated just by the letter W. For clarity, five regions of the spectrum are enlarged.

nt = 8, 40C, 120C, 1024C, sw NOE = 7000 Hz, sw H = 5500 Hz and a mixing time of 200 ms).

^{1}H, ^{15}N and ^{13}C chemical shifts were referenced directly (^{1}H) and indirectly (^{15}N, ^{13}C) to the frequency of DSS, using the absolute frequency ratios (Markley et al., 1998). Spectra were processed using NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed for resonance assignment using NMRView (Johnson and Blevins, 1994). The backbone assignments were confirmed through the observation of the inter-residue NOEs expected from the sequential connectivity.

Extent of assignments and data deposition

The protein consists of 306 residues, including 15 prolines. The extend of ABP assignments is: 94% of ^{1}H and ^{15}N backbone amides, 94% of ^{13}Cα, 92% of ^{13}Cβ and 93% of ^{13}C′. The missed assignments belong mainly to two short segments comprising residues 16–19 and 100–103. Residues 67 and 255 are located between proline residues and hence were not assigned. Assignments were done with the help of the program MAPPER (Güntert et al., 2000). A secondary structure prediction using the chemical shift index method for Cα, Cβ and C′ atoms was performed (Wishart and Sykes, 1994). This prediction is in good agreement with the crystal structure (Quiocho et al., 1989). The ^{1}H, ^{15}N and ^{13}C chemical shifts for ABP have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6948.

Acknowledgements

A.H.D. acknowledges a postdoctoral fellowship from the Ministerio de Ciencia y Tecnologia (Spain). A.C. acknowledges the support of a Wellcome Trust Travelling Research fellowship (U.K.). This work was supported by BBSRC (grant no. 24/B12992) and is a contribution from the North of England Structural Biology Centre.

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