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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of the rat liver fructose-2,6-bisphosphatase domain

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Biological context

The bifunctional enzyme, 6-phosphofructo-2-kinase/ fructose-2,6,-bisphosphatase (PFK-2/FBP-2), provides a switch between the glycolytic and gluconeogenic pathways in liver (Okar et al., 2001). This regulation is accomplished by way of fructose-2,6-bisphosphate (F-2,6-P_2). Because PFK-2/FBP-2 both makes and degrades F-2,6-P_2, the bifunctional enzyme controls the cellular content of this crucial biofactor. The regulatory power of this system has been exploited to achieve significant improvement of glycemic control in rodent models of Types 1 and 2 diabetes mellitus (DM) (Okar et al., 2001; Wu et al., 2002).

The crystal structures of both the rat testis PFK-2/FBP-2 (PDB code 1BIF), as well as a rat liver bisphosphatase domain (PDB code 1FBT) from which the N-terminal PFK-2 domain had been deleted, have been determined (Hasemann et al., 1996; Lee et al., 1996). The structures confirmed the spatial separation of the two active sites, as had been predicted from the primary sequence. When considered as a distinct enzyme, the FBP-2 domain belongs to the superfamily of phosphoglycerate mutase-like proteins (Winn et al., 1981). The success of targeting hepatic F-2,6-P_2 content as a means to treat DM underscores the relevance of the sequential resonance assignments of the rat liver FBP-2 domain (residues 250-470 of the bifunctional enzyme) that we report in this letter. These data provide a crucial step in the continued use of NMR spectroscopy to understand the function of hepatic FBP-2.

Methods and experiments

The separate rat liver FBP-2 domain (residues 250-470 of the bifunctional enzyme) was expressed in BL21(DE3) *E. coli* cultures and purified by the methods reported previously (Okar et al., 1997). Global labeling of the protein with ^{13}C and ^{15}N was accomplished by including 99% uniformly ^{13}C-labeled glucose and 98% u-[^{15}N] ammonium sulfate in the minimal growth medium (Cambridge Isotope Laboratories, Andover, MA). Partial deuteration was accomplished by including 90% D_2O (Isotech, Miamisburg, OH) in the expression cultures and pre-conditioning the starter cultures with D_2O. Purified protein was exchanged into the final buffer by ultrafiltration. Protein samples were greater than 95% pure by SDS/PAGE analysis and no significant degradation was observable during the acquisition of NMR experiments. The enrichment levels for stable isotopes in the protein samples were greater than 95% for ^{15}N and ^{13}C, while ^{2}H enrichment was near 80%. NMR were either 2.7 mM ^{15}N, ^{13}C-labeled bisphosphatase or 1.5 mM ^{2}H, ^{15}N, ^{13}C-labeled protein in 100 mM sodium phosphate pH 7.0, 1 mM EDTA, 0.02% NaN_3 in 90% H_2O/10% D_2O. No additional signals from an ^{15}N-^{1}H-HSQC acquired from protonated FBP-2 (data not shown) were detected when compared to the corresponding spectrum of deuterated protein.

The NMR experiments were performed at 25 °C on Varian Unity INOVA 600 and 800 MHz spectrometers (doubly-labeled protein) as well as, a Bruker Avance DMX 800 MHz and an Avance 600 MHz spectrometer equipped with an ^{1}H, ^{2}H, ^{13}C, ^{15}N-tunable (TXI) cryogenic probehead (triply-labeled protein). Proton chemical shifts were referenced to internal DSS. Indirect referencing was used for ^{15}N and ^{13}C chemical shifts. All spectra were processed with nmrPipe/nmrDraw (Delaglio et al., 1995) and analyzed in NMRView (Johnson & Blevins, 1994). Linear prediction was used in all indirectly detected dimensions of 3D experiments. Sequential assignments were obtained from a series of 3D experiments:

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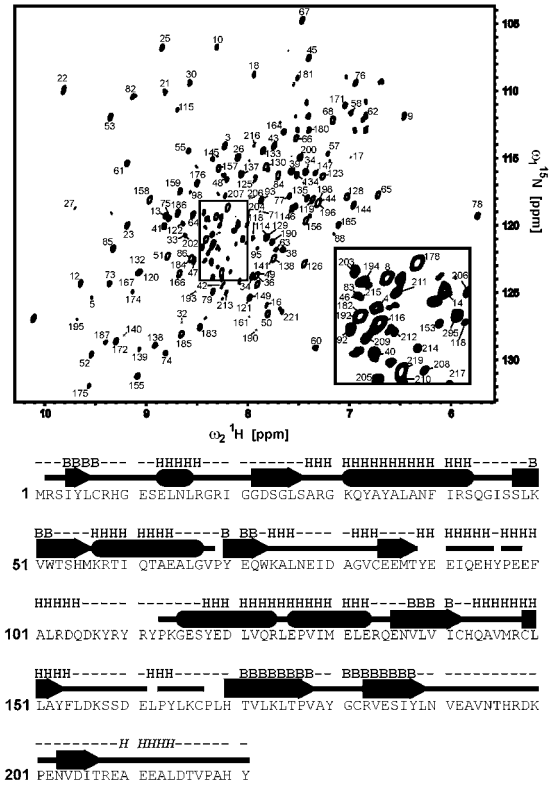

Figure 1. 2D ^{1}H-^{15}N-HSQC of globally ^{2}H, ^{13}C, ^{15}N-labeled fructose-2,6-bisphosphatase. Assigned residues are indicated by their sequence number within the separately expressed FBP-2 domain; the sequence position within the bifunctional enzyme is calculated by adding 249 to the FBP-2 domain sequence number. The boxed region is shown expanded in the inset for easier visualization. Signals that are not visible in this HSQC are indicated by an asterisk. The amino acid sequence, together with the predicted secondary structural elements obtained from the consensus chemical shift index, are also shown; β-strands are indicated by arrows and α-helices by bars. Residues involved in β-strands and α-helices in the crystal structures of rat liver FBP-2 (Lee et al., 1996) and rat testis PFK-2/FBP-2 (Hasemann et al., 1996) (italicized) are denoted by B and H, respectively.

HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH (Cavanagh et al., 1996). In addition some side-chain carbons could be assigned with the help of a CCH-TOCSYNNH. For all NH-detected experiments TROSY versions (Pervushin et al., 1997) were used and ^{2}H-decoupling was employed for spectra acquired from fractionally deuterated protein.

Extent of assignments and data deposition

Figure 1 shows the ^{1}H, ^{15}N-HSQC with assignments of triply-labeled rat liver FBP-2 domain. Despite the large size (221 residues), backbone assignments (N, NH, CA, CB, CO) were obtained for 96% of all residues outside the flexible loop between Y96 and Y112. Sidechain carbon assignments were possible only for a few residues located mainly in the highly flexible C-terminal region. Fast T_2 relaxation prevented sidechain assignments for most residues with the insensitive CCH-TOCSYNNH and related experiments. Secondary structural elements obtained through the consensus chemical shift index (CSI) are in general agreement with the X-ray structure of FBP-2 domains (Figure 1) (Hasemann et al., 1996, Lee et al., 1996). Those regions of the domain for which the CSI and X-ray data do not fully agree are regions that have also been implicated as having a role in the function of PFK-2/FBP-2.

It is noteworthy that large differences in signal intensities were observed in all spectra, which we attributed to differences in transverse (T_2) relaxation times. Narrow, strong signals, indicative of higher flexibility, have been found in the C-terminal 21 residues (P201-Y221) as well as several loops near the active site in the crystal structure (Y164-V172, Q44-W52, L16-L26). More detailed studies of the protein dynamics within the FBP-2 domain are currently in progress. The chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 7573.

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References

Cavanagh, J. et al. (1996) *Protein NMR Spectroscopy*, Academic Press, San Diego.
Delaglio, F. et al. (1995) *J. Biomol. NMR*, **6**, 277–293.
Hasemann, C.A. et al. (1996) *Structure*, **4**, 1017–1029.
Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.
Lee, Y.H. et al. (1996) *Biochemistry*, **35**, 6010–6019.
Okar, D.A. et al. (1997) *Protein Expr. Purif.*, **11**, 79–85.
Okar, D.A. et al. (2001) *Trends Biochem. Sci.*, **26**, 30–35.
Pervushin, K. et al. (1997) *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 12366–12371.
Winn, S.I. et al. (1981) *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **293**(1063), 121–130.
Wu, C. et al. (2002) *Am. J. Physiol. Endocrinol. Metab.*, **282**, E38–45.