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Letter to the Editor: Sequence specific ^{1}H, ^{13}C and ^{15}N resonance assignments of the hath-domain of human hepatoma-derived growth factor

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Biological context

Hepatoma-derived growth factor (HDGF)-related proteins (HRPs) comprise a new protein family displaying mitogenic activities toward various cell lines. The first member of HRPs, human HDGF (hHDGF) was discovered in the conditioned medium of human hepatoma cell line and was shown to stimulate DNA synthesis in Swiss 3T3 cells (Nakamura et al., 1994). Recently, several more proteins in this family, including mHRP-1, mHRP-2, hHRP-3, bHRP-4, and p52/75/LEDGF, were found to display mitogenic activities toward other different cell lines, such as HuH-7 cells, HEK-293 cells, fibroblasts, endothelial cells and smooth muscle cells (Izumoto et al., 1997, Everett et al., 2000). HRPs were found to contain a highly conserved N-terminal domain of $\sim$ 100 residues called the 'hath' domain (homologous to the amino terminus of HDGF) (Dietz et al., 2002). The hath domain has been speculated to have a function common to all HRPs, but C-terminal region, which vary in lengths and charges in different HRPs may play specific function in various organs (Dietz et al., 2002).

hHDGF is a 240 a.a. protein with a molecular mass of 27 kDa. It has been found in various compartments, including in secretions, in the nucleus of smooth muscle cells, and in the cytoplasm of rat metanephrogenic mesenchymal cells (Everett et al., 2000). In addition, hHDGF has been shown to play a role in nephrogenesis, tumorgenesis, and the development of vascular, kidney and liver (Matsuyama et al., 2001). Similar to other growth factor, hHDGF binds to heparin and heparan sulfate with K_d values of around 37 and 16 nM, respectively. The binding is specific and cannot be attributed to the negative charge of heparin since HDGF does not bind to chondroitin sulfate. The observation provides the prospect that HDGF, similar to fibroblast growth factor (FGF) and endothelial growth factor (EGF) may be able to interact with heparin or heparan sulphate on the cell surface. The interaction with heparin might facilitate the internalization and nuclear targeting.

hHDGF shares high sequence homology with the high mobility group (HMG) protein. However, hHDGF is believed to be functionally and structurally distinct from HMG-1 proteins (Nakamura et al., 1994). Furthermore, hHDGF is the first of a group of over thirty-nine proteins identified to contain a PWWP motif (Stec et al., 2000). The PWWP domain spans some 70 amino acids and it is present in proteins of nuclear origin and play a role in cell growth and differentiation. The exact function of this motif is still unknown but it was hypothesized to be involved in protein interactions and/or DNA binding (Stec et al., 2000). Although two 3D structures related to PWWP domain were already elucidated (Qiu et al., 2002, Slater et al., 2003), the sequence homology between these two PWWP domains and that of hath domain is relatively low and no structure of the hath domain has been reported. In order to gain insight into the structure-functional relationship of hHDGF and related HRPs we have applied NMR techniques to determination the structure of hHDGF. In this letter we report the complete resonance assignments of hHDGF hath domain.

Methods and experiments

The N-terminal hath domain of hHDGF corresponding to amino acids 1-100 was constructed into plasmid, pET6H (modified from pET11d, Novagen), with an N-terminal 10 residue His-tag, $M(H)_6AMA$ for a total length of 110 residues. ^{13}C, ^{15}N-labeled NMR samples were prepared from *E. coli* strain,

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BL21(DE3) harboring DNA construct and grown in M9 minimal medium supplemented with $^{15}NH_4Cl$ (1 g/L), ^{13}C glucose (1 g/L), and ^{15}N, ^{13}C-labeled CELTONE (0.5 g/L, Spectra Stable Isotopes). The culture was grown with 0.1 mg/ml ampicillin at 37 °C to $OD_{600} \sim 0.6$ and induced by 1 mM isopropyl-1-thio-β-D-thiogalactoside (IPTG) for another 4 h. The cells were harvested by centrifugation, resuspended with lysis buffer (300 mM NaCl, 10 mM imidazole, and 50 mM Na_2HPO_4, pH 8.0), and ruptured by microfluidizer. Soluble protein in the supernatant of cell lysate was allowed to bind to Ni^+-NTA agarose (Qiagen) and eluted with 250 mM imidazole. The proteins were further purified to homogeneity by size exclusion chromatography using a Sperdex 75 column (Amersham Biosciences) in 10 mM phosphate buffer, 150 mM NaCl, 0.01% NaN_3, and 1 mM EDTA at pH 7.4. The purity was found to be better than 95% by Coomassie Blue-stained PhastGel (Amersham Biosciences). The samples containing 2 to 3 mM proteins in 100 mM phosphate buffer, 150 mM NaCl, 0.01% NaN_3, and 1 mM EDTA in 95% H_2O/5% D_2O at pH 6.0 are ready for NMR measurements.

All the NMR data were acquired at 25 °C on 500 MHz and 600 MHz Bruker AVANCE spectrometers equipped with a TXI quadruple probe (^1H, ^{13}C, ^{15}N, ^{31}P). Sequential backbone resonance assignments for ^1HN, ^{15}N, $^{13}C_\alpha$ and $^{13}C_\beta$ were derived from HNCA, HN(CO)CA, HNCO, HN(CA)CO, CBCANH, and CBCA(CO)NH experiments (Bax and Grzesiek, 1993). The assignments of H_α and H_β were achieved from HBHA(CO)NH experiment. ^{15}N-edited TOCSY (mixing time 80 ms), H(CC)(CO)NH, CC(CO)NH and HCCH-TOCSY were used for side chain assignment. Gradient selection was carried out in all described 3D experiments. NMR data processing was achieved by using XWINNMR and Aurelia software (BRUKER) on a SGI workstation. The ^1H chemical shift was referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) at 0 ppm. The ^{15}N and ^{13}C chemical shift values were referenced using the consensus ratio Ξ of 0.101329118 and 0.251449530 for $^{15}N/^1H$ and $^{13}C/^1H$, respectively (Wishart, 1995).

Extent of assignments and data deposition

The ^1H-^{15}N-HSQC spectrum of hHDGF, together with the assignments of the resonances, is shown in Figure 1. The excellent resonance dispersion indicated that the hath domain has ordered structure. Complete backbone assignments and more than 95% side chain assignments were achieved. The chemical shift data have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu/) under the accession number 5902.

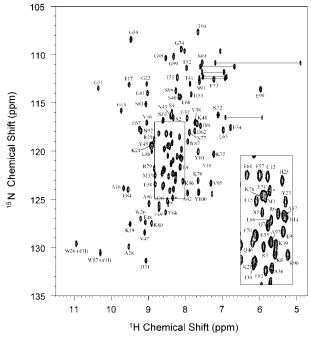

Figure 1. 600 MHz ^1H-^{15}N HSQC spectrum of hHDGF hath domain obtained at pH 6.0 and 25 °C. Assignments of the backbone resonances are also shown.

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